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EPIDEMIOLOGY OF KAPOSI SARCOMA IN A SOUTH AFRICAN ADULT BLACK POPULATION

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A THESIS

Submitted to the Faculty of Health Sciences, University of Witwatersrand, in

fulfilment of the requirements for the degree of

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2022

Declaration

I [Melitah Motlhale] declare that this thesis is my own original, unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any other University or institution.



26.09.2022

Melitah Motlhale Date

Dedication

This thesis is dedicated to my amazing parents, Mr Nonofo Motlhale and Mrs Banyana Montirisi Motlhale, my wonderful siblings Mr Temo Motlhale, Miss Abaleng Goyang Motlhale and Miss Basadi Kelebogile Motlhale. I am deeply grateful for your love and support.

In the loving memory of my late supervisor, Dr Elvira Singh, my late grandmother, Mrs Baratang Botshome and my late uncle, Mr Eagile Mokaunyana Botshome. Pity you left before I completed this degree. To say I miss you would be an understatement, thank you for the life lessons. I will forever be grateful for your love and wonderful memories. I will always love you.

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List of Abbreviations

ADCs	AIDS defining cancers
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked immunosorbent assay
GLOBOCAN	Global Cancer Incidence, Mortality and Prevalence
hCG	human chorionic gonadotropin
HHV-8	Human herpesvirus-8 (KSHV)
HIV	Human Immunodeficiency virus
HIV-1	Human Immunodeficiency virus type 1
IARC	International Agency for Research on Cancer
ICD-O-3	International Classification of Disease for Oncology 3rd edition
ICD-10	International Classification of Diseases 10th Revision
IFA	Immunofluorescence assay
IQR	Interquartile range
IRIS	Immune reconstitution inflammatory syndrome
JCS	Johannesburg Cancer Study
KS	Kaposi sarcoma
KSHV	Kaposi sarcoma-associated herpesvirus (HHV-8)
LANA	Latency-Associated Nuclear Antigen

NADCs	Non-AIDS defining cancers
OR_{adj}	Adjusted odds ratio
OR	Odds ratio
PCR	Polymerase chain reaction
PhD	Doctor of Philosophy
PLWH	People living with HIV
SA	South Africa
SSA	sub-Saharan Africa
VEGF	Vascular endothelial growth factor

Thesis material

Original publications

Paper 1: **Motlhale M**, Sitas F, Bradshaw D, Chen WC, Singini MG, de Villiers CB, Lewis CM, Muchengeti M, Waterboer T, Mathew CG, Newton R and Singh E. Epidemiology of Kaposi's sarcoma in sub-Saharan Africa (*Published, Cancer Epidemiology 78 (2022) 102167*).

Student's contribution to publication: Student conducted a literature search, conceptualised the manuscript, data extraction and analysis, conducted data analyses, drafted the manuscript, revised the manuscript and submitted the manuscript to the journal.

Co-authors' contribution: Provided guidance on the conceptualisation of the objectives, editing of the manuscripts and/or reviewing of the manuscript drafts.

Paper 2: **Motlhale M**, Sitas F, Bradshaw D, Chen WC, Mwiza Gideon Singini M.G, de Villiers CB, Lewis CM, Muchengeti M, Waterboer T, Mathew CG, Newton R and Singh E. Lifestyle factors associated with sex differences in Kaposi sarcoma incidence among adult black South Africans: A case-control study (*Published, Cancer Epidemiology 78 (2022) 102158*).

Student's contribution to publication: Student conceptualised the paper, perfomed data analyses, drafted the manuscript, revised the manuscript and submitted the manuscript to the journal.

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Paper 3: Motlhale M, Muchengeti M, Bradshaw D, Chen WC, Singini MG, de Villiers CB, Lewis CM, Bender N, Mathew CG, Newton R, Waterboer T, Singh E, and Sitas F. Kaposi sarcoma-associated herpesvirus, HIV-1 and Kaposi sarcoma risk in black South Africans diagnosed with cancer during antiretroviral treatment rollout (*Submitted to International Journal of Cancer*).

Student's contribution to publication: Student conceptualised the paper, conducted data analyses, drafted the manuscript, revised the manuscript and submitted the manuscript to the journal.

Co-authors' contribution: Provided guidance on the conceptualisation of the objectives, editing of the manuscripts and/or reviewing of the manuscript drafts.

Thesis Abstract

Background

Kaposi sarcoma (KS) is a malignant neoplasm caused by infection with Kaposi's sarcoma associated herpesvirus (KSHV). KS is a common AIDS-defining cancer in sub-Saharan Africa. KS was endemic in South Africa even before the advent of the human immunodeficiency virus (HIV). There is a consistent male predominance of KS incidence before and after the advent of HIV. The risk of KS is amplified in HIV-immunosuppressed individuals, and antiretroviral therapy (ART) reduces KS incidence. Literature on the epidemiology of KS in adult black Africans is sparse, concentrating mainly on cancer risk in relation to KSHV and HIV among African populations. Reliable data on the relationship between these factors, especially in explaining the uneven sex ratios are lacking in African populations. The overall aim of this PhD is to investigate the epidemiology of Kaposi sarcoma in an adult (18-74 years) black South African population.

Methods

Firstly, we describe the trends in incidence of KS in sub-Saharan Africa before and after antiretroviral rollout periods using the best available KS incidence data from WHO-International Agency for Research on Cancer (IARC) and the African Cancer Registry Network (AFCRN) publications.

Data for the second and third part of the thesis came from the Johannesburg cancer study (JCS), a cancer epidemiological biobanking study of over 25,000 cancer patients recruited between 1995 and 2016 who provided answers to a short questionnaire and a blood sample. The JCS is particularly suited to answer multiple associations via case-control comparisons. Secondly, we

assessed the prevalence of known cancer modifying factors (parity, hormonal contraceptive use in females, sex-partners, urban/rural status, education, smoking and alcohol consumption in both sexes) in relation to KS. Thirdly, using sera that were tested for HIV-1 and KSHV antibodies, we measured the association between KSHV seropositivity and select sociodemographic variables, measured the association between KS and seropositivity to HIV-1 and KSHV and assessed KSHV seropositivity before (1995-2004), early (2005-2009) and late (2010-2016) ART rollout periods. We calculated case-control adjusted odds ratios (OR_{adj}) using unconditional logistic regression to assess these relationships and especially whether any of these could account for the unequal KS sex ratios.

Results

Using data from 16 registries in 14 countries and a subset with some KS trend data, an increase in KS incidence was observed in four out of 16 registries throughout sub-Saharan Africa as the HIV epidemic progressed, reaching peak incidences pre-ART rollout. Between early and late rollout periods 10 out of 16 registries showed a decline in KS incidence. The overall unweighted average decline in KS incidence between early and late ART rollout periods was 27%.

In the second study, 1,275 cases and 10,309 infection unrelated controls were included, with particular attention to selecting controls that are not associated with exposures of interest. We found a positive association between KS and heavy vs. non-drinking (OR_{adj}=1.31;95%CI=1.03–1.67), and in current heavy vs. never smokers (OR_{adj}=1.82;95%CI=1.07–3.10).

In the third study, 1,237 KS cases and 6,649 infection unrelated cancer controls were included. KSHV seropositivity among 1,237 KS cases was 98%. Among 6,649 controls, KSHV seropositivity was higher in males (OR_{adj}=1.4;95%CI=1.23–1.52), in persons with HIV, (OR_{adj}=4.2;95%CI=3.74–4.73) and lower in high school leavers (OR_{adj}=0.7;95%CI=0.59–0.83). The risk of KS among those with HIV was high, with no difference in risk between the sexes (OR_{adj}=86.96;95%CI=60.36–125.29, $P_{heterogeneity}$=0.934). Among those who were KSHV seropositive the risk of KS was also high with no difference in risk between males and females (OR_{adj}=132.21;95%CI=86.30–202.52, $P_{heterogeneity}$=0.297). Compared to HIV-1 and KSHV seronegatives, KSHV seropositives yielded an adjusted OR for KS of 26;(95%CI=11–62) in HIV-1 seronegative participants and increased 100-fold to an adjusted OR of 2501;(95%CI=1083–5776) in HIV-1 seropositive participants. KSHV seropositivity declined over the ART rollout periods (37%, 28%, 28% P_{trend}<0.001) but also coincided with increases in high school leavers over the same periods (46%, 58% and 67%, P_{trend}<0.001). HIV-1 seroprevalence increased from 10% pre-ART period to 22% in late ART period (P_{trend}<0.001).

Conclusions

ART rollout coincides with a decline in KS incidence across several regions in sub-Saharan Africa. There is a need to establish enduring and accurate cancer registration in most regions of Africa. The role of other risk factors in the published literature could not be fully ascertained. We show that smoking and alcohol consumption may provide a possible explanation for the KS sex differences, given both exposures are more common in men, but given this is the first report of such an association in a case-control study, further, preferably cohort studies are needed to confirm this association. HIV-1 increases risks of KS in those infected with KSHV by 100-fold. Even

though these modern serological assays for KSHV are highly sensitive in detecting KSHV, they are not specific in a high KSHV prevalence setting such as South Africa. Decline in KSHV seroprevalence coincide with ART rollout and with improvements in educational standards and general hygiene. Despite the decline in KS, it remains common among males and females in South Africa. The role smoking and alcohol play in relation to viral loads of HIV/KSHV, differences in immunological responses or other genetic differences between males and females warrant further studies. Further studies are also needed to improve the clinical utility of the current KSHV serological assays by improving their specificity.

Thesis layout

PART 1: GENERAL INTRODUCTION

- ***Chapter 1****: Background of the study and Literature review*.

- ***Chapter 2****: Methodology*.

PART 2: EMPIRICAL CHAPTERS

- ***Chapter 3****: Epidemiology of Kaposi's sarcoma in sub-Saharan Africa (Published).*

- ***Chapter 4****: Lifestyle factors associated with sex differences in Kaposi sarcoma incidence among adult black South Africans: A case-control study (Published).*

- ***Chapter 5****: Kaposi sarcoma-associated herpesvirus, HIV-1 and Kaposi sarcoma risk in black South Africans diagnosed with cancer during antiretroviral treatment rollout (Under review).*

PART 3: GENERAL DISCUSSION

- ***Chapter 6****: Discussion and Conclusion*.

PART 1: GENERAL INTRODUCTION

Chapter 1: Background of the study and Literature review

This chapter provides background information on KS in South Africa, sub-Saharan Africa and globally. The literature review includes cancer burden, the epidemiology of KS, the role of KSHV in the epidemiology of KS, KS pathogenesis, KS in males and females, HIV and KS in South Africa and the effect of ART on KS. The relevance and justification, overall aim and objectives of this study are also given.

1.1 Background

The global cancer burden has been rapidly increasing (1,2). The number of new cancer cases increased from 7.6 million to 12.7 million between 2002 and 2008 (2). Because of expected demographic changes, cancer cases are estimated to continue to rise at 70% per year over the next 20 years (3). From 2008 to 2020 new cancer cases increased from 12.7 million to 19.3 million (1,2). There is substantial variation of cancers attributed to infection between geographical regions, from 3.3% in Australia to sub-Saharan Africa shouldering the majority of the burden at 33% (1 in 3) (4–6). Cancer burden is rapidly growing worldwide and this could be attributed to the complex reasons reflecting aging, growth of the population, distribution of risk factors for cancer associated with socioeconomic development (7).

Infections with certain viruses, bacteria and parasites have been identified as causal risk factors for certain cancers (8). Eleven infectious agents have been classified as Group I human carcinogens by International Agency for Research on Cancer (IARC) (9). These include Human Immunodeficiency virus (HIV) and Kaposi sarcoma-associated herpesvirus (KSHV) or Human

herpesvirus-8 (HHV-8), among others (4–6). HIV is regarded as causal for a number of cancers (including KS), it causes cancer through immunosuppression, thereby enhancing the carcinogenic action of other viruses like KSHV (9) and also directly activates oncogenes through the HIV Tat proteins (6,10–12). Globally, infection attributed cancers are likely to become an important complication as a result of people living longer with HIV due to access to antiretroviral therapy (ART) (13). Although people infected with HIV in sub-Saharan Africa constitute about 70% of the global HIV/AIDS epidemic (14,15), the effect of HIV on cancer burden in sub-Saharan Africa is still partially described.

This thesis focuses on describing the epidemiology of KS, one of the leading cancers as a result of the HIV/AIDS epidemic. Saliva is considered as the major route of transmission of KSHV from infected adults to children, in sub-Saharan Africa and there is a high prevalence in the adult population (16–18). In South Africa the prevalence KSHV infection in adults ranges from 16% to 75% using different methods of detection (19–21). The risk of KS is significantly high among individuals infected with HIV and KSHV (19). The relationship between KSHV seropositive, HIV and the occurrence of KS in the South Africa population is not well understood. The effect of ART on the burden of KSHV and HIV and their impact on KS is still unclear.

KS is endemic in a number of countries in sub-Saharan Africa and infrequent globally, in 2020 GLOBOCAN reported about 34,000 cases and 15,000 deaths (2). Before the introduction of ART, one of the first expressions of HIV infection among patients was KS (22). Prior to the advent of the HIV/AIDS epidemic, KS was present in sub-Saharan Africa at a larger frequency than the rest of the world (23–25). Until the 1980s, little focus had been accorded to KS, which was regarded

as an indolent tumor with relatively slow clinical progression (26). With the emergence of the HIV/AIDS epidemic, the clinical severity, incidence and mortality of KS increased significantly, having a profound impact in the region (26–28). KS burden is greatest in sub-Saharan Africa and this could be attributed to the endemicity of KSHV as well as the high HIV burden in the region. Even though sub-Saharan Africa has a huge burden of HIV/AIDS, data on trends of KS and other HIV/AIDS related malignancies is limited (29).

KS is a common cancer among people living with HIV (PLWH) (30,31). The likelihood of developing KS among PLWH in South Africa is around 50-134 fold higher (27,28). Adult males are at higher risk of developing KS than females, and by contrast ratios in children are about the same (23,32). In South Africa, between 1988 and 2017, the pathology-based National Cancer Registry reported that KS incidence rates doubled in males and increased about seven-fold in females. This resulted in a decline of the sex ratio from 7:1 (males versus females) in 1988 to 2:1 in 2017 (20,33,34). Even though the ratio has declined, the KS sex differences are still evident.

Literature on the contribution of HIV to the etiology and pathogenesis of KS in adult black Africans is sparse, concentrating mainly on cancer risk in relation to KSHV and HIV among African populations, with small sample sizes (19,27,35). Little is known about the epidemiology of KS in adult black South Africans. Therefore this study seeks to describe the epidemiology of KS among adult black South Africans, to assess the effect of ART rollout on KS in sub-Saharan Africa, to assess whether the lower incidence of KS in females could be attributed to any of known cancer-associated lifestyle factors and measure the background seroprevalence of KSHV antibodies and measured the relationship between HIV and KSHV antibodies seropositivity and

KS among black South Africans using data from a large epidemiological cancer study (N=26,263) in Africa.

1.2 Literature review

1.2.1 Cancer burden

Cancer cases continue to rise globally and currently estimates indicate that there will be approximately 1.27 million new cancer cases and 1 million deaths by 2030; with the developing countries being contributors of more than 80% of the new cancer cases in the world (3). KS was reported as the second largest contributor to cancer burden in sub-Saharan Africa in 2012 (5,36). Cancer is an emerging critical public health issue in South Africa and the percentage of all deaths ascribed to cancer in South Africa has gradually increased over the past few years from 8.7% in 2014 to 9.3% in 2016 (37,38). Cancer inflicts economic expenses on individuals and society (39). The economic expenses include lost productivity, because of premature death due to cancer as the society loses the individual's contribution to the economy (40). Unless addressed, the increasing cancer burden could counteract the gains made from stabilizing some infectious and communicable disease rates in South Africa and increasing life expectancy (41).

1.2.2 Kaposi sarcoma

Moritz Kaposi first described KS in 1872 (42–44). KS is a multifocal tumor that manifests in four distinct epidemiological forms. Prior to the HIV/AIDS epidemic, an indolent form was common among elderly males from the Mediterranean, Eastern European descent and Middle Eastern heritage (classic KS or sporadic KS) (45). In the 1950s, physicians increasingly recognized a florid and more aggressive form of KS in sub-Saharan Africa specifically in Central and East Africa

(endemic KS) (23–25,43). In the 1970s a form of KS manifested in organ transplant recipients under immunosuppressive treatment (iatrogenic KS). The most recent form of KS was reported early 1980s in patients with the HIV/AIDS (AIDS-related KS or epidemic KS) (45). In the United States of America this highly aggressive form of KS was commonly reported among young men who had sex with men (45,46). HIV-1 infection is commonly linked to epidemic KS than HIV-2 infection (45). In sub-Saharan Africa the burden of epidemic KS increased as the HIV/AIDS epidemic progressed (47). Epidemic KS was often spread, progressed rapidly and frequently deadly (48).

In sub-Saharan Africa KSHV is endemic and HIV is widespread (49). KS remains one of the leading cancers in PLWH in sub-Saharan Africa, accounting for significant morbidity and mortality (1,31). The clinical presentation and the course of disease for KS vary according to the different types, the histological appearance of skin lesions in all types of KS is similar (42). KS often presents as cutaneous lesions on the body (24,50). The lesions usually present on the skin, mucous membranes lining the nose, mouth, throat or viscerally in lymph nodes or other internal organs (51). KS lesions on the skin are painless and do not itch. So often the patient does not think much of them and may not report them to the doctor/clinician and this contributes to late presentation. The HIV clinician therefore must be proactive in seeking KS lesions on patients. KS lesions can be hyperpigmented, spanning from brown to black in Africans to other shades include red, pink and purple, depending on the patient's skin colour (47,51). The absence of cutaneous lesion on the skin does not exclude visceral KS (24). KS is often diagnosed based on the appearance of lesions and diagnosis is usually confirmed by histology (45,52).

It is difficult to screen patients for KS because unlike cervical or bowel cancer, there is no pre-neoplastic stage of KS for which screening can be performed (45,53). The exception is when epidemic KS presents in oral cavity as the first affected anatomic site, in such cases screening could benefit the patients (54). Early detected KS prognosis has better clinical outcomes compared to late detection (45). Currently there is no prophylactic vaccine against KSHV infection in clinical settings. However, treatment options for KS include the use of ART.

1.2.3 KSHV

KSHV was discovered in 1994 (55). KSHV is a double stranded DNA virus from *Herpesviridae* gamma subfamily (10). The development of KS lesions among individuals infected with HIV without KS is reported to be predicted by the detection of KSHV in peripheral blood cells of the patients (48,56). The risk of developing KS increases with increasing antibody titer to KSHV and the risk is greater among those who tested positive for HIV compared to those who were HIV negatives (33).

In Africa, KSHV is transmitted mainly orally via saliva, although other routes such as sexual, or blood-borne may play varying roles (57). Evidence for vertical transmission of KSHV is less apparent and seems to be mostly horizontal (58). Healthy individuals can be infected with KSHV without any complications. KSHV has been consistently linked to KS as a necessary cause, sufficient evidence also shows that KSHV causes primary effusion lymphoma and multicentric Castleman's disease (4,9,10,13,19,33,47,59).

The seroprevalence of KSHV is approximately 20-80% in Africa and the Mediterranean region. It is slightly more than 30% among black South African hospital patients in comparison to less than 10% in more developed countries (27,33,57,60). KSHV seroprevalence rates were higher in the black South African population living in the rural areas (21). Seroprevalence of KSHV has been found to be the same among males and females but increasing with increasing age (33). KSHV seroprevalence decrease with increasing level of education and this proposes that factors related to poverty may facilitate the transmission of KSHV (19,33). The level of education is often used as an indicator for socio-economic status. Poor socio-economic status increases risk of KSHV infection (61,62). KSHV seroprevalence roughly reflects the incidence of KS in a given population, there are other important cofactors that contribute in the development of KS (63).

Prevention of KSHV infection would prevent KS and other KSHV associated tumors (48). Currently there is no primary prevention or a vaccine against KSHV (24) and theoretically it should be feasible to develop an effective KSHV vaccine (48). Data on seroprevalence of KSHV in South Africa is sparse and this calls for further research to increase understanding of the epidemiology of KS in the local context and inform the feasibility of developing an effective KSHV vaccine.

1.2.4 KS pathogenesis

A proportion of KSHV seropositive individuals develop KS, this suggests that other factors contribute to the development of KS. HIV is a significant co-factor in the pathogenesis of KS (56,62,64,65). Immunodeficiency increases the risk of developing KS among KSHV seropositive individuals (49,56,63). Further cofactors such as genetic, immunologic, and environmental must be necessary for the development of KS by interacting with KSHV, affecting the immune system

or other indirect reasons (63,66). Pathogenesis of KS in PLWH has been connected to immune suppression and cytokine dysregulation (29). KSHV establishes a life-long infection in the host that depends upon virus-encoded immune evasion genes and genes that influence cellular proliferation, survival, migration, angiogenesis and cytokine or chemokine production (49).

The lytic and latent phrases of the KSHV cycle play significant roles in the pathogenesis of KS. In the lytic phase, entry, uncoating, and nuclear import are followed by a coordinated sequence of viral transcription, DNA replication, and assembly, followed by the final release of nascent virions and a latent life phase (49). Latent genes include latency-associated nuclear antigen (LANA), kaposins A, B and C, viral cyclin (v-cyclin) and v–Flice-inhibitory protein (v-FLIP) (67,68). Lytic genes include, K1, K8, K3, K5, viral interleukin-6 (vIL-6), vIRF-1, viral macrophage inflammatory proteins (vMIPs), vBcI-2, viral G-protein-coupled receptor (vGPCR) and K15 (68,69).

K8 protein plays a role as transactivation repressor for ORF50 protein, leading to a negative autoregulation system during lytic infection (68). LANA plays a principal role in viral replication of the latent viral episome (49,69). LANA expression is necessary for persistent infection through inhibition of apoptosis and maintenance of latency. Because all KSHV-infected cells express LANA-1, it is a useful for diagnosis of KSHV infection (67). Proteins expressed during latency and lytic infections can be useful antigens for the sensitive detection by serum antibodies from KS patients (69).

Angiogenesis is one of the most important characteristics of epidemic KS (70). Vascular endothelial growth factor (VEGF) and its receptors are pro-angiogenic factors and contribute significantly in the pathogenesis of KS (65,70,71). Spindle-shaped epidemic KS cells expressed increased amounts of VEGF compared to HIV-1 positive cells without KS (65). The VEGF upregulation is promoted by several KSHV genes such as, K1, LANA and vGPCR (71,72). KS is a pathogenetically and morphologically diverse disease but the clinical significance of this heterogeneity is poorly understood (73).

1.2.5 KS in males and females

KS is predominant in males than females (20,23,32,33,74). The reasons for the male predominance are still unknown. The difference between males and females is much more evident among HIV negative adult patients with predominance ranging from 10 to 20:1 (74,75). In the early 1980s, KS was a rare disease, the incidence was 2 to 3 fold higher in men than women globally, the incidence increased 20 fold since the onset of HIV/AIDS epidemic in sub-Saharan Africa (45). The epidemiology has changed dramatically since epidemic KS was first diagnosed in the 1980s in South Africa (73). In South Africa, the incidence of KS has risen at least three fold between 1988 and 1996 (20,47). There is a need to explore whether the sex differences are related to any modifying factors like parity, number of sexual partners, hormonal contraceptive use, smoking and alcohol consumption.

1.2.6 HIV-1 and KS in South Africa

There are two types of HIV that are relevant to the HIV epidemic, HIV-1 and HIV-2. HIV-1 is responsible for approximately 95% of HIV infections globally. HIV-1 is divisible into ten sub-

types (A-H, J, K) and a few circulating recombinant forms are recognized, HIV-1 subtype C accounts for about half of all global HIV-1 infections (76). HIV infection, predominantly through immunosuppression, results in enhanced replication of oncogenic viruses (77–79). Southern Africa is home to approximately a third of all PLWH worldwide and South Africa has one of the largest HIV epidemic, globally (27). HIV infected individuals are predominately blacks and it affects more females than males (80).

Several cancers are recognized as being connected to immunosuppression in PLWH (13). Historically, the occurrence of KS, in a person infected with HIV has constituted an AIDS diagnosis and is classified as one of the AIDS defining cancers (ADCs) (13,27,81). In sub-Saharan Africa the estimated age standardized incidence rate (world) of KS was reported to be ≥0.56 per 100 000 and age standardized mortality rate (world) of ≥0.19 per 100 000 in all sexes in 2020 (**Figure 1-1 and Figure 1-2**).

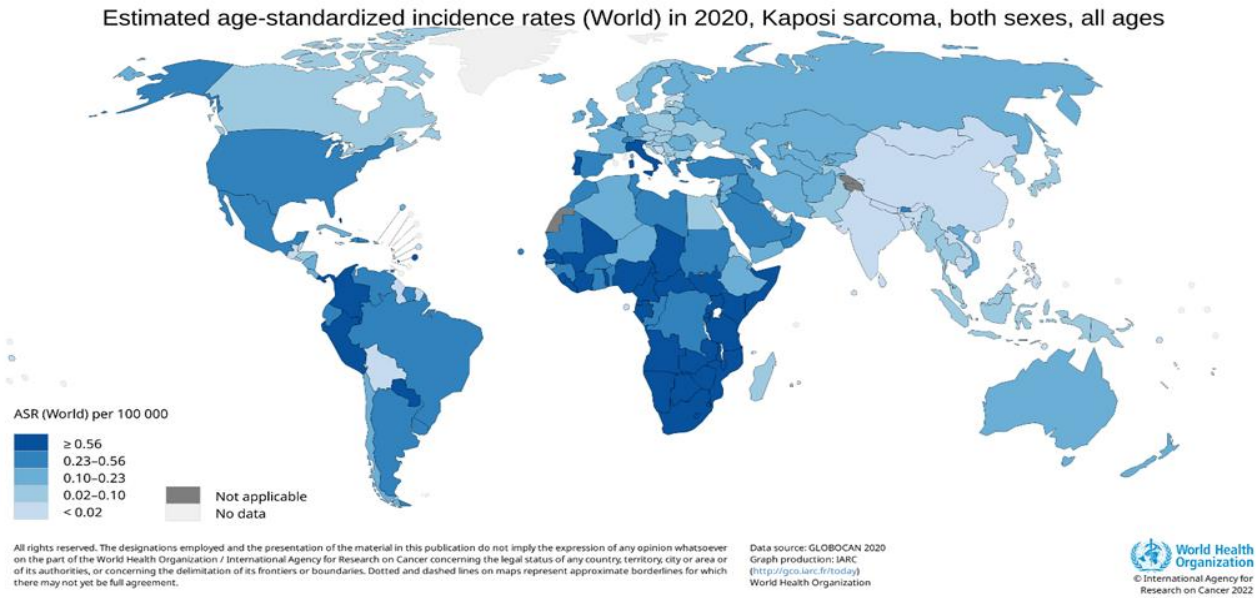

Figure 1-1: Kaposi sarcoma age standardized incidence rates in 2020 (https://gco.iarc.fr/today/online-analysis-map).

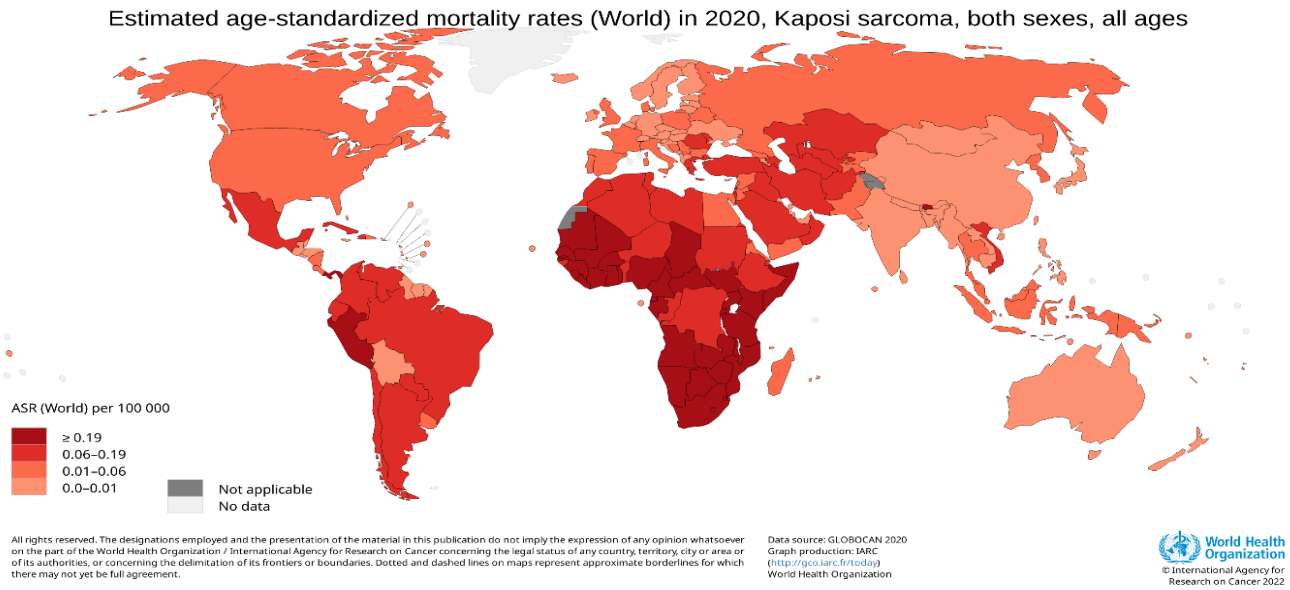

Figure 1-2: Kaposi sarcoma age standardized mortality rates in 2020 (https://gco.iarc.fr/today/online-analysis-map)

1.2.7 ART and KS

The HIV/AIDS epidemic dynamics are changing due to the use of ART. The scale up of accessibility to life prolonging ART in sub-Saharan Africa through different programs, has notably improved the survival rate of patients with HIV infection, this is most likely to impact the range and burden of cancer among PLWH (78,81). South Africa has the biggest ART program in the world accounting for 20% of individuals on ART globally, since its rollout in 2004 (80,82). South Africa is the first country in Africa to approve use of antiretroviral medicine for HIV prevention. In South Africa Pre-exposure prophylaxis (PrEP) was introduced in 2016 and prioritized for sex workers and in 2017 to include additional at-risk group including young women, adolescent girls and men who have sex with men (83). The ART coverage was reported at approximately 57% in 2015 and it increased to approximately 62% in 2018 (84–86). Gaps in the ART program may also contribute to the burden of KS. Data from developed countries indicated significant decreases in

incidence of KS among PLWH on ART therapy (10,78). The association between HIV and KS in relation to the advent of ART at population level is worth investigating in the African context since data from sub-Saharan Africa is scarce.

KS was relatively rare in South Africa compared to central African countries before the HIV/AIDS epidemic (23), but its incidence increased significantly as the epidemic progressed (27,75). The pathology based data from the South African National Cancer Registry data show an age adjusted incidence rate of KS in 1988 per 100,000 of 0.06 in all females and 0.4 in all males (87). The age adjusted incidence rates peaked at 2.79 and 4.69 in 2004 (at the time when ART rollout started in South Africa) and has since decreased slightly to 2.58 and 4.45 per 100,000 in 2014 among black females and black males respectively (88,89). One study on interrupted time series analysis of the impact of ART on KS incidence in South Africa using histologically confirmed surveillance data from the South African-National Cancer Registry for the years 1999 to 2016, reported that the introduction of the ART was associated with a decrease of over 50% in the predicted age-standardized incidence rates of KS (90). The decline in KS incidence is promising, but nevertheless the role of ART on the HIV/KSHV/KS relationship has not been fully described in South Africa.

The timing of introduction to ART is very important on the outcome of KS management. KS survival is predicted by the stage at which KS is diagnosed, in South Africa it was reported that patients with late stage KS had a two-fold higher mortality than individuals with early disease (45,91). Patients with KS need to be diagnosed and treated with ART earlier to decrease the mortality. ART has been associated with better prognosis and prolonged life of PLWH, including the reduction of KS when diagnosed and treated early and proper adherence is observed

(47,48,73,92–95). Before ART coverage was increased across sub-Saharan Africa, most epidemic KS patients were diagnosed very late and rarely survived (96). ART is necessary in prevention of the development of KS among PLWH and in reduction of morbidity and mortality attributed to KS in sub-Saharan Africa (94).

ART regimen may result in complete remission the KS lesions within a few weeks or months post starting ART among patients presenting with limited cutaneous lesions (45,49). In other cases patients with advanced disease, the initiation of ART can result in immune reconstitution inflammatory syndrome (IRIS) (49,66). Globally the incidence of epidemic KS decreased since the introduction of ART. However, KS continues to occur in PLWH and cases are still being identified among patients on ART (45,48). With scale up of ART in sub-Saharan Africa, PLWH continue to live longer with an increase in life expectancy. The impact of ART on the incidence of epidemic KS in sub-Saharan Africa is difficult to quantify because fewer data are available (45,95) and this calls for further research.

Final Remarks

South Africa is characterized by a high HIV prevalence and KSHV is endemic, so there is a need to understand the epidemiology of KS in a local context. Infectious diseases can often be prevented and/or treatable therefore, cancers attributed to infectious agents could be prevented (10). There is need to establish effective, affordable, feasible cancer prevention and control programs to limit the epidemic of KS.

1.3 Conceptual framework

The conceptual framework was informed by literature reviewed on herpesvirus seroprevalence, herpesvirus-related cancers, HIV infection and related topics, see **Figure 1-3** below. A previous framework, developed to shed some light on the causes of oral cancer, was adapted (97), and restricted to data that has already been collected. The original framework included information on modifiable, not modifiable and emerging factors that were directly linked to cancer. For this study, modifiable factors include alcohol consumption, smoking, lifetime number of sexual partners, parity, contraceptive use, and ART and level of education. Non-modifiable factors include sex, age, ethnicity, place of birth and place of residence. The potential risk factor is HIV.

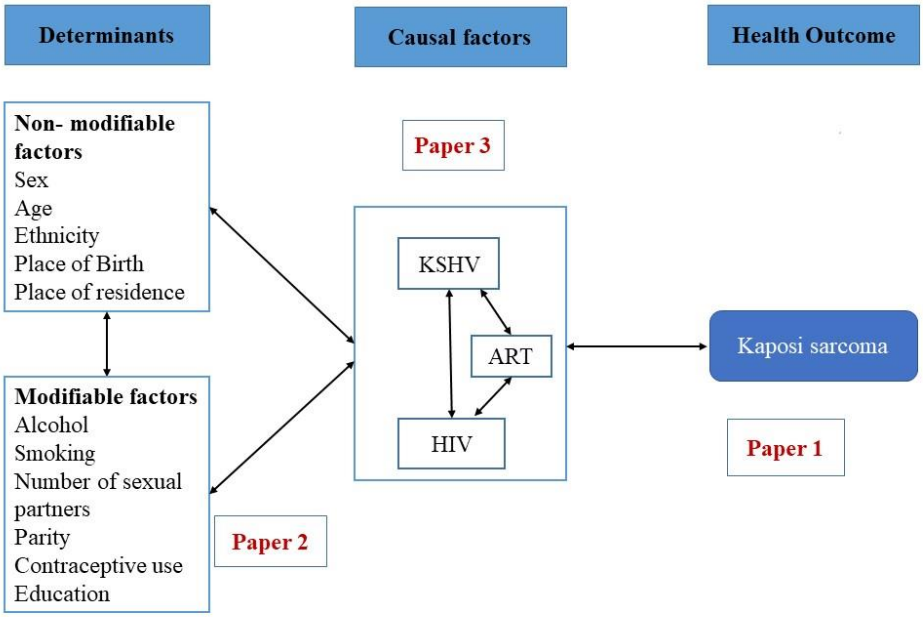

Figure 1-3: Conceptual framework for investigating the epidemiology of Kaposi sarcoma.

1.4 Rationale

Cancer is an emerging critical public health and economic issue in South Africa (37,98). There is lack of recent local data on the role of HIV on KS in African populations. The African populations make up more than 67% of the PLWH (81). The data available on KS is from developed countries with low HIV and KSHV seroprevalence, thus not reflective of the local context (13,78). Available risk factor data on KS cancers in South Africa is from over 10 years ago and these early studies employed small sample sizes (19,27,33,62,75,99). The Johannesburg Cancer Study (JCS) covers a sufficient number of cancer cases pre and post ART rollout to explore the epidemiology of KS.

Literature on the effect of ART on KS in South Africa where the ART was rolled out nationally in 2004 is limited (82). There are differences in HIV epidemic dynamics, the endemicity of KSHV, different cancer specific risk factors and population variations between developing and developed countries. These differences warrant exploration of KS in relation to HIV in South Africa.

Research on different infectious agents linked to specific cancers is of paramount importance. A large potential exists for reducing the cancer burden caused by infections (5). Hepatitis-B associated liver cancer, for example, has been declining in incidence because of effective vaccination programs and screening of the blood supply (100). The findings from this study can inform policy, interventions for prevention, management and control of KS in South Africa.

1.5 Gaps in the literature

KS trends

There are pronounced deficit in cancer registration in this region, characterized by low population coverage, poor data quality and notable urban bias. Continued investment of research into HIV associated malignancies in sub-Saharan Africa countries can catalyse a broad cancer care and research agenda, which will be beneficial to PLWH and HIV negative individuals.

Effect of ART on KS

There is a need to evaluate the ART effects in the quest to improve the overall outcomes among PLWH. The continued recording of high numbers of KS in sub-Saharan Africa this might be attributed to the ART treatment gap in the region.

Sex ratio differences

It is not clear why in KSHV endemic countries, when the virus is equally prevalent in males and females why KS is much more frequent in males.

KSHV seroprevalence

The most effective cancer prevention strategy is primary prevention, and this should be integrated with early diagnosis and appropriate therapies. There is lack of information on the seroprevalence of KSHV in sub-Saharan Africa and its clinical utility in detecting early KS.

1.6 Relevance and justification

This study area is one of the neglected research areas in cancer studies in the South African context despite its importance. A lot is still unknown on KS in general. The literature review has revealed that there were few studies in Africa on this topic. This study utilised data from the largest cancer

epidemiological study in Africa, thus providing more accurate estimates of seroprevalence of KSHV. The study estimated the seroprevalence of KSHV by demographic characteristics. A previous study on this topic utilized 51 cases of KS (19), the current study consisted of 1,275 cases of KS, and provides far more detail on the epidemiology of KS than previously reported. Background seroprevalence of KSHV is extensively studied in the local context. Seroprevalence is important as it is an indicator of cumulative exposure to KSHV adult black South African population.

The study investigated the associations between KSHV, relation to HIV on the risk of developing KS in much detail using high throughput serological assays. The previous HHV-8 assay was a labour-intensive Immunofluorescence Antibody Assay (IFA). The modifying effect of ART on KSHV and KS was also assessed. None of this has been studied in such detail in the black population. For example, identifying viral and lifestyle factors that explain the uneven KS sex differences will inform interventions on actionable targets for prevention and treatment. South Africa has one of the largest HIV pandemics worldwide. KSHV is endemic in South Africa. HIV prevalence in South Africa is high among the black population and it affects more females than males. In contrast, in developed countries where most of the studies on KS were done the most affected sub-population groups were white men. This study adds to the body of knowledge on the epidemiology of KS among blacks in the South African context.

Lastly, even though ART program was rolled out in 2004 coverage is at approximately 57% and 62% in 2018. These gaps may also contribute to the variation on the burden of KS between the developed countries that rolled out ART in the early 1990s with better ART coverage and the

South African context. There are differences in the HIV epidemic dynamics, the endemic KSHV, different cancer specific risk factors and population variations between the developing countries and the developed countries. The current study utilizes person-level data (as opposed to ecological studies – e.g., Majaya et al (90) – which reported a decrease in age-standardised incidence rate of KS over time), with serological confirmation of KS and HIV, lifestyle factors, large sample size, spans over 21 years, including pre-ART, and during the different ART periods – to access of person-level risk factors of KS in the evolving South African HIV epidemic. These numbers of KS, duration of study, and the laboratory investigations are not readily available in many high HIV and high KS burden areas (which are often Low- and Middle-income countries), so this study offers a unique opportunity to zoom into the South African experience of KS and HIV. There is a paucity of literature on this topic and this study will fill this gap. There is need for more research on KSHV, HIV, and KS in the South African context prior to considering targeted prevention and control policies.

1.7 Research aim and objectives

1.7.1 Overall aim

To investigate the epidemiology of Kaposi sarcoma in the adult (18-74 years) black South African population.

1.7.2 Specific objectives

Using data from the JCS of incident black South African cancer patients aged 18-74. This study was a secondary data analysis of the JCS comprising 1,275 patients with KS and approximately 10, 309 controls (objective 2 and 3), we used World Health Organization-International Agency for

Research on Cancer (IARC) and the African Cancer Registry Network (AFCRN) reports to evaluate the effect of ART on KS in sub-Saharan Africa (objective 1) the study objectives were:

1. To evaluate the KS incidence rates and assessed the effect of ART on KS in Sub Saharan Africa (**Paper1**).
2. To assess whether the lower incidence of KS in females than males is related to any of the lifestyle factors (e.g. number of sexual partners (male and female), hormonal contraceptive use and parity (females only), smoking and alcohol consumption) (**Paper 2**).
3. To evaluate the relationship between KSHV, HIV and KS and to assess the seroprevalence of KSHV among infection unrelated study controls, during antiretroviral treatment rollout (**Paper 3**).

Chapter 2: Methodology

This chapter provides information on the methodology used in this study. It comprises the study design, study population, data sources, selection criteria, study variables, data management and analyses plan, and ethical considerations. Data for this study was obtained from the JCS (objective 2 and 3) which is the largest case control cancer study in in Africa. The study was established in 1995 and ended in 2016. We used the WHO-IACR and AFCRN reports to evaluate the effect of ART on KS in sub-Saharan Africa (objective 1).

2.1 Study design

This study was an extensive data analysis of an existing case control study; the JCS under the auspices of the South African National Cancer Registry. The original study objectives of the JCS were to obtain up-to-date, robust estimates on the role of key lifestyle factors on leading and emerging cancers in adult black South African population, and secondly, to examine with serology, the role of known or suspected oncogenic infections on cancer risk including infectious agents like HIV. Lastly, (funding permitting) the study aimed to identify susceptibility loci for leading cancer types and loci associated with immune response to infection.

2.2 Study population

The JCS was established in 1995 to redress at that time the historical paucity of epidemiological cancer research among black South Africans. The aims of the JCS were to examine whether key known and emerging risk factors for cancer in Western countries (i.e. mainly white populations) applied to black patients locally, in Johannesburg, South Africa. These aims evolved into measuring the relative importance of known and emerging risk factors for cancer in a local setting

and establishing a biobank and the largest study on the continent for ongoing investigations of infectious and genetic drivers of cancer. Another objective of the JCS is to identify susceptibility loci for leading cancer types among blacks as most studies were done among Caucasians (whites). The JCS is similar to a hospital-based case-control study. By recruiting patients with any cancer, the JCS is amenable to multiple case-control comparisons. Simply, cases comprise the cancer types of interest and controls are a subset of the other cancer types that are not associated with the exposures of interest. This text-book approach to control selection for each analysis is explained in detail in each relevant chapter.

2.3 Data sources

Objective 1

We extracted and tabulated from available WHO-IARC publications and the AFCRN National HIV prevalence and ART coverage data were derived from the Joint United Nations Program on HIV/AIDS (UNAIDS)/WHO.

Objectives 2 and 3

The JCS recruited consecutive consented, treatment-naïve adult, self-defined Black male and female incident cancer patients of any type arising mainly from southern Gauteng province, attending the medical and radiation oncology departments of the largest tertiary referral public hospitals in Johannesburg (mainly Charlotte Maxeke hospital), between 1995 to 2016 (27). Over 95% of cancers were confirmed by pathology (27). Cancer types were coded to topography and morphology using the International Classification of Diseases for Oncology Version 3 (ICD-O3) and presented in ICD-10 format.

2.3.1 Questionnaire

Trained nurse interviewers used a standard questionnaire to interview participants in their preferred language (Zulu or Sotho). Participants were interviewed less than 6 months after diagnosis. The three-page questionnaire covered questions on the leading and emerging risk factors for cancer. Questions included place of birth and residence, education, home language of parents, method of cooking and heating, smoking by type of tobacco and amounts smoked, snuff use, alcohol consumption by type, parity, use of oral and injectable contraceptives, number of sex partners, basic occupations (**Appendix 1**), and self-reported use of ART (since 2005) (**Appendix 2**).

2.3.2 Serological data

Blood samples were collected in one serum separation tube and one EDTA vacutainer before chemotherapy or radiation therapy, thus avoiding any potential DNA or protein damage of the bio-samples. Serum samples were aliquoted and stored at -25°C. Blood samples were tested for HIV-1 locally and shipped in dry ice and tested in Heidelberg, Germany using a multiplex immunofluorescence assay for presence of 20 infectious agents that are known, or are suspected to cause cancer, including the herpesviruses 1-5 and HHV-8/KSHV. All laboratory analyses were carried out with personnel blinded to individual sample characteristics by only issuing a sample ID number. Demographic and cancer type results were matched to the laboratory results.

Serological assays results were used to compare the frequencies of seropositivity and immunofluorescent levels of antigens of the infectious agents. Frequencies and immunofluorescent levels were previously shown to be related to KSHV viral load (a proxy to

viral or bacterial load in some infectious agents) (19). The multiplex serology assay is a glutathione S-transferase (GST) capture immunosorbent assay utilizing fluorescent-bead technology, as previously described (101). Recombinant GST fusion proteins of viral and bacterial antigens expressed in bacteria are loaded on different colour glutathione-casein-coupled fluorescence-labeled polystyrene beads (SeroMap, Luminex, Austin, TX) and affinity-purified. These uniquely colour-labeled beads are loaded with different proteins, mixed and incubated in 96-well plates with an equal volume of serum diluted in pre-incubation buffer to a final 1:1,000 dilution. Antibodies bound to the beads via the GST fusion proteins are stained with biotinylated goat anti-human immunoglobulin and the reporter conjugate R-phycoerythrin-labeled streptavidin.

A Luminex 200 analyzer identifies the bead colour and thus the specific antigen carried by the bead. The quantity of bound antibodies is determined as the median fluorescence intensity (MFI) of at least 100 beads per antigen per sample. Reproducibility of multiplex serology is assessed using 180 duplicate samples tested each assay day. We analyzed the serum for antibodies against two KSHV antigens (LANA and K8.1).

2.4 Selection criteria

Participants aged 18 to 74 years, black, resident of South Africa, with a new (incident) diagnosis of cancer provided they have not begun chemotherapy or radiation treatment and had given written or witnessed oral, informed consent were included in the analyses. ‘‘Cases’’ for this study are participants with a recently diagnosed KS. Controls consist of participants diagnosed with cancer types that have no established relationship to infectious agents. Control selection was carefully thought through especially when assessing the effect of potential risk factors on KS e.g. smoking

(smoking-related cancers were excluded), alcohol consumption (alcohol-related cancers were excluded) and hormonal contraceptive use (contraceptive-related cancer were excluded).

2.5 Study variables

For this study, the main outcome variable was KS. The main exposure variables were lifestyle and demographic factors previously known to be associated with cancer (mainly smoking, alcohol, reproductive factors and KSHV). The main stratification variables were HIV, ART period and sex. Other covariates (confounders) include age, education, parity, place of residence, parity, lifetime number of sexual partners.

2.6 Statistical analysis

Research question 1: Is the KS incidence rate changing post ART in sub-Saharan Africa?

KS incidence data were extracted and tabulated from available WHO-IARC publications and the AFCRN. National HIV prevalence and ART coverage data were derived from UNAIDS/WHO. Data on ART were gathered per sub-Saharan Africa country together with available HIV prevalence and available KS incident data. We reviewed published English articles on KS and ART related studies to assess the incidence of KS and the effect of ART over time in sub-Saharan Africa. This review reflects on KS trends pre- and post-ART in sub-Saharan Africa.

Research question 2: **Is the lower incidence of KS in females than males related to any of the lifestyle factors (e.g. number of sexual partners (male and female), hormonal contraceptive use and parity (females only), smoking and alcohol consumption)?**

Unconditional unmatched logistic regression was used to calculate case-control adjusted odds ratios (OR_{adj}), and 95% confidence intervals (95%CI) of developing KS and each of the modifying factors, using appropriate comparison controls. Controls were cancer types that had no known relationship to exposures of interest (infection or alcohol or smoking or contraceptive use).

Research question 3: What is the relationship between KS and seropositivity to HIV and KSHV antibodies and the seroprevalence of KSHV pre- and post-ART roll out periods in black South Africans.

The seroprevalence of the KSHV and any associations with other socio-demographic variables was assessed basic frequency tabulations. The infection unelated control group was used to estimate the seroprevalence of KSHV in the background population, pre- and post-ART. We used a case-control approach and unconditional unmatched logistic regression models to calculate OR_{adj} and 95% CI between KS in relation to KSHV and HIV, and KSHV prevalence before and after ART rollout.

2.7 Ethical considerations

Anonymity and confidentiality were maintained throughout the study, the study participants were identified by unique identification number. The University of Witwatersrand Human Research Ethics Committee (Medical) approved the primary study. Ethics clearance for this study was obtained from the University of the Witwatersrand Human Research Ethics Committee (Certificate no: M191130 – **Appendix 3**).

PART 2: EMPIRICAL CHAPTERS

Chapter 3: Epidemiology of Kaposi sarcoma in sub-Saharan Africa

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This chapter presents a narrative review on the epidemiology of KS and assessed the effect of ART on KS in sub-Saharan Africa. We describe the incidence of KS in sub-Saharan Africa, in four time-periods, (1) before 1980 (before HIV/AIDS era); (2) 1981-2000 (early HIV/AIDS era, limited or no ART coverage); (3) 2001-2010 (early ART coverage period); and (4) 2011-2016 (fair to good ART coverage period). HIV prevention programs and good coverage of ART in developed countries resulted in a remarkable decline in HIV-KS incidence and better KS prognosis. By contrast, in sub-Saharan Africa, population ART rollout has lagged, but clinical studies have shown positive results in reduction of KS incidence and better KS prognosis. However, the effect of ART rollout in relation to population KS incidence is unclear. Data for this study was obtained from the WHO-IARC publications and the AFCRN. National HIV prevalence and ART coverage data were derived from UNAIDS/WHO. The original paper publication has also been included in **Appendix 4**.

3.1 Background

KS is a malignant neoplasm caused by infection with KSHV (31). Prior to the advent of the HIV/AIDS epidemic, KS was present in Sub Saharan Africa at a larger frequency than the rest of the world (23–25). Until the 1980s, little focus had been accorded to KS, which was regarded as

an indolent tumor with relatively slow clinical progression (26). With the emergence of the HIV/AIDS epidemic, the clinical severity, incidence and mortality of KS increased significantly, having a profound impact in the region (26–28). The impact included increases in incidence in younger age groups, a narrowing of the male-female ratio, and an increase in KS into regions where it was not endemic (20,33). KS is now predominantly an AIDS defining malignancy and one of the commonest cancers in sub-Saharan Africa.

Epidemic KS has been described due to coinfection with HIV and KSHV. KSHV is a necessary cause of KS (9,26). The seroprevalence of KSHV in Africa is relatively high (20-80%) (27,33,60). sub-Saharan Africa accounts for approximately 70% of the global burden of HIV infection (102,103). Co-infection with HIV and KSHV greatly increases the likelihood of developing KS (19). Being male and age are factors related with risk of KS where it affects older people in endemic and classical, younger people in epidemic KS. Studies have further hypothesized that (non-viral) demographic and lifestyle risk factors for all KS types include infection with helminths and alcohol consumption (19,29,109–111,47,55,96,104–108). The role of ethnicity in the etiology of KS is geographically bound by latitude north and south of central-east Africa and seems to be even less relevant in the presence of HIV than it was for the development of the classical or endemic KS before the HIV pandemic (20). In the absence of HIV infection, risk factors associated with endemic KS remain unclear, this includes the male to female difference (96).

In sub-Saharan Africa, ART is used to manage HIV / and AIDS related conditions including epidemic KS (96). Since the introduction of ART, KS clinical outcomes have improved significantly with a combination of ART and chemotherapy (30). In sub-Saharan Africa, complete

remission of KS has been reported in about 20-80% of patients who were ART compliant (92,94). The median time for remission in patients with KS due to ART has been reported to be between three to nine months (48). ART is a cost effective intervention in decreasing the burden of KS, and offers great potential for improvements on survival, and quality of life for those with HIV-associated KS (52). ART has also been shown to be an important way to reduce the risk of developing KS among PLWH in high income countries both in clinical follow-up and population studies (95). The HIV burden, late rollout of ART, socio-economic issues and intervention implementation challenges in sub Saharan Africa could possibly explain the differential intervention outcomes between high income countries (102,112). Clinical studies from Kenya, Uganda and South Africa indicate that ART is effective in reducing the incidence of KS (29,94,95).

In most sub-Saharan African countries, public sector ART programs were rolled-out in the mid-2000s. By 2017 approximately 11.8 million (46%) people living with HIV or AIDS in sub-Saharan Africa were receiving treatment (30). ART coverage estimates are regularly compiled by the most recent report received by WHO and / or UNAIDS. The ART coverage estimates are calculated using statistical modelling methods (1).

WHO updated the ART guidelines for adults and adolescents in 2009. All adults and adolescents, including pregnant women with HIV infection and a CD4 count at or below 350 cells/mm^3 were enrolled for treatment, regardless of whether or not they have clinical symptoms. Those with WHO clinical stage 3 or 4 were to start treatment, irrespective of CD4 cell count. Old guideline thresholds for treatment initiation was <200 cells/mm^3 (113). In 2016, WHO launched revised, more inclusive guidelines for ART eligibility (30). Since 2017, all people living with HIV or AIDS are

recommended to receive ART, irrespective of CD4 count (30,114). Most sub-Saharan African countries are formally rolling out ART programs, and most programs are now widening eligibility criteria to cover a broader group of HIV infected individuals outside of hospital systems.

There have been several review papers on KS pre and post the advent of HIV/AIDS (circa 1980) covering incidence or relative frequency of KSHV, associated risk factors in sub-Saharan Africa countries, and descriptions of the epidemiological and clinical characteristics of different forms of KS (29–31,96,115). One review in 2010 focused on the epidemiology and transmission of KSHV as well as the clinical characteristics and therapy of HIV-associated KS in sub-Saharan Africa, with concerns raised about ART population coverage (93). However, the effect of ART rollout on KS in sub-Saharan Africa at a hospital or population level has not been systematically documented.

We hypothesized that ART rollout should cause a decline in population-based incidence of Kaposi sarcoma in sub-Saharan Africa by comparing KS incidence to time periods pre and post rollout of ART.

3.2 Methods

This is a synthesis of literature on KS incidence and trends in relation to ART rollout in sub-Saharan Africa. We used KS incidence data available from WHO-IARC publications and the AFCRN. National HIV prevalence and ART coverage data were derived from UNAIDS/WHO. Data on ART were gathered per sub-Saharan Africa country together with available HIV prevalence and available KS incident data. We reviewed published English articles on KS and ART related studies to assess the incidence of KS and the effect of ART over time in sub-Saharan

Africa. Countries from North Africa were excluded because their climate conditions and cultural backgrounds have been considered to be more Mediterranean than African (1). Conference abstracts were excluded. This review reflects on KS trends pre- and post-ART in sub-Saharan Africa.

3.2.1. KS age standardized incidence rates

KS incidence and trend data were derived from sub-Saharan African cancer registry reports in IARC Cancer Incidence in Five Continents (CI5) Volumes I-XI, two IARC Monographs on Cancer in Africa (116–118) and cancer registry reports published by the AFCRN (119,120). KS age standardized (world) incidence rates (ASIR) per 100 000 persons were summarized for four time-periods, (1) before 1980 (before HIV/AIDS era); (2) 1981-2000 (early HIV/AIDS era, limited or no ART coverage); (3) 2001-2010 (early ART coverage period) and (4) 2011-2016 (fair to good ART coverage period). Cancer registry data published by IARC and AFCRN were classified as being population-based i.e. representing reasonable quality cancer registries and a good indication of KS incidence thus appearing in CI5 series (CI5 Vol VIII (1993-1998) (116), CI5 Vol X (2003-2007) (117) and CI5 Vol XI (2008-2012) (118)) and those published by AFCRN (Cancer in Africa Epidemiology and Prevention (1960-2000) (119), Cancer in Africa (2001-2014) (121) and Cancer in sub-Saharan Africa AFCRN Vol III (120). GLOBOCAN reports (GLOBOCAN 2008 and 2012 reports (1,122), which in sub-Saharan Africa interpolate several countries' data, were only used for regional summary statistics.

3.2.2. Adult (15-49 years) HIV/AIDS prevalence, ART rollout and ART coverage percentage

Adult HIV prevalence data were drawn from country specific UNAIDS/WHO epidemiological fact sheet 2004 updates (123). ART rollout and coverage data were drawn from data provided by UNAIDS/WHO website(85,86).

3.2.3 Regions

Data are presented for each country and within five African regions as defined by the United Nations Population Division (1). Northern (excluded here for reasons mentioned previously, and for not falling in sub-Saharan Africa), Eastern, Southern, Middle (Central) and West Africa.

3.2.4 KS trends

Trends of the ASIR of the incidence of KS over the four time periods were presented graphically for males and females (**Figure 3-1** and **Figure 3-2**). **Figure 3-3** illustrates the relationship between ART coverage and decline in KS incidence between 2001 and 2010 (early ART coverage period) and 2011-2016 (fair to good ART coverage period).

3.3 Results

Registries from eight of the 46 sub-Saharan African countries appeared in CI5 volumes I-XI and AFCRN reports and they were classified as fairly good quality registry data. Cancer registration coverage per region was poor with one country reporting out of the eight countries in Southern Africa, four countries out of the 14 countries in Eastern Africa, no country reporting in Central Africa (eight countries) and three countries reporting out of 16 West African countries.

3.3.1. ART Coverage (2018)

ART coverage increased from late 1990s, early 2000s to 2018, 30 countries have high (above 50%) ART rollout rates by 2018. In 2018 the lowest ART coverage was reported in Madagascar (9% in 2018) and the highest in Namibia at 92% (85,86).

3.3.2. Adult HIV prevalence, and ART roll out

The adult prevalence of HIV varies across sub-Saharan Africa, with a prevalence of <0.1% in Comoros and 27.3% in Eswatini in the year 2018. Southern Africa has the highest burden of HIV compared to other regions. The earliest national rollout of ART in sub-Saharan Africa was in 1998 (Senegal); most countries rolled out ART in early 2000s and later programs started in 2006 (Chad).

3.3.3 Reported KS ASIR incidence per 100 000 from before 1980 to 2016

Table 3-1 reports the countries that reported KS ASIR to CI5. Before 1980, two population-based registries; Zimbabwe (Bulawayo) and Uganda (Kampala) reported KS incidence data for this period. Between 1981 and 2000, six registries reported KS cases and were published in CI5: Malawi, France La Reunion, Uganda (Kyadondo), Zimbabwe (Harare), The Gambia, and Mali (Bamako). For the period between 2001 and 2010, seven registries reported KS cases and were published in CI5: South Africa (Eastern Cape), Malawi, Uganda (Kyadondo), Zimbabwe (Harare), The Gambia, Mali (Bamako) and Nigeria (Ibadan). Seven registries published KS cases in CI5 between 2011 and 2016: South Africa (Eastern Cape), France La Reunion, Uganda (Kyadondo), Zimbabwe (Bulawayo), Zimbabwe (Harare), The Gambia and Mali (Bamako).

Table 3-2 reports on KS age standardized incidence rates (ASIR) for sub-Saharan African countries for 4 time-periods (pre-1980, 1981-2000, 2001-2010 and 2011-2016). **Figures 3-1** and **3-2** shows KS ASIR trends over the four time periods. KS incidence rates varied over 10 to 20-fold amongst countries, from 2.6 to 39.3 in males and 0.3 to 21.8 in females; pre-1980, 0.0 to 32.0 in males and 0.0 to 11.8 per 100 000 in females for 2011-2016. In the pre-1980 period, KS was so rare that it was often combined with skin cancer or other miscellaneous categories or not classified in cancer registry reports (116).

3.3.3.1 Southern Africa

In the Southern African region, there was a lack of data on incidence rates of KS pre-1980. In 2008, GLOBOCAN reported that the KS incidence rates (per 100 000) for Southern Africa were 11.5 and 5.1 among males and females, respectively. GLOBOCAN 2012, reported a decrease on KS incidence rates (7.6 and 4.7 among males and females respectively). High incidence rates were reported in Swaziland/Eswatini [1981-2000]; males 17.2 and females 9.5. The ASIR decreased to 13.5 among males and 6.4 in females by 2011-2016. In Namibia, the incidence rates were lower for the 1981-2000 period [6.8 males, 1.9 females], there was a pronounced increase in the 2001-2010 period [23.2 males, 7.7 females].

3.3.3.2 Eastern Africa

Before 1980, only two registries (Uganda and Zimbabwe) out of the 14 countries in Eastern Africa reported KS age standardized incidence rates to IARC. There are still large gaps in data availability with no data reported for some countries. GLOBOCAN 2008 and 2012 reports indicate that this region shoulders most of the KS burden in sub-Saharan Africa. For 2001-2010, the ASIR for males

was 14.9 and 6.8 for females and the rates increased to 15.1 for males and 7.6 for females [2011-2016]. Mozambique and Zimbabwe reported high incidence rates. In Mozambique (Beira), the ASIR for KS was 63.6 for males and 27.5 for females [2001-2010] and decreased to 32.0 for males and 11.2 females [2011-2016]. In Zimbabwe (Harare), the KS incidence rate was high 50.8 for males and 20.8 for females [1981-2000]. The reported rates decreased to 37.3 for males and 23.5 for females [2001-2010] and 18.4 for males and 10.3 for females [2011-2016].

3.3.3.3 Central Africa

GLOBOCAN reported that the KS ASIR for Central Africa were 4.1 and 0.6 among males and females respectively in 2008. GLOBOCAN 2012, reported a decrease on KS incidence rates (1.2 and 0.4 among males and females respectively). The only country that reported over all time periods was Congo (Brazzaville) and was characterized by a decrease in KS incidence rates over time. KS incidence varied from 4.3 for males and 1.4 for females [1981-2000] to 0.6 for males and 0.4 for females [2001-2010] and during 2011-2016 the ASIR for males was 0.5 and 0.0 for females.

3.3.3.4 West Africa

This region reported the lowest KS ASIR and the rates decreased over time. The ASIR was 1.9 for males and 1.2 for females [2001-2010] and the rates decreased to 0.9 for males and 0.6 for females [2011-2016] according to the GLOBOCAN reports in 2008 and 2012. Mali (Bamako) reported KS rates ranging from 2.1 for males and 1.0 for females [1981-2000] to 1.7 for males and 0.7 for females [2001-2010] and during 2011-2016 the ASIR declined for males to 0.8 and to 0.6 for females.

In summary, in the 16 registries from 14 countries that had sufficient serial data on KS, two showed no increase or decrease, 10 showed a decline and four showed an increase in KS incidence between 2000 and 2010 (early ART coverage period) and 2011-2016 (fair to good ART coverage period). GLOBOCAN reports on regional KS incidence estimates which vary from country to country within each region. With the understanding that these data are limited, incomplete and "ecological", **Figure 3-3** illustrates the geographical association between percentage ART coverage (latest data available in 2018) and KS incidence in the early vs. more recent ART periods. The overall unweighted average decline in KS incidence between the early ART and later period was 27%, but this decline was not statistically significant across the region. **Figure 3-4** illustrates the relationship between the latest adult (15-49) HIV prevalence (%) and KS incidence between 2011 and 2016. There was a moderate positive correlation between HIV prevalence and KS incidence for males and females.

Table 3-1: Countries that reported KS ASIR to C15 pre- and post- HIV/AIDS epidemic.

Country ASIR published in CI5	**Pre- and post- HIV/AIDS epidemic**			
	Before 1980	**1981 – 2000**	**2001 – 2010**	**2011 – 2016**
Zimbabwe	✓	✓	✓	✓
Uganda	✓	✓	✓	✓
Malawi		✓	✓	
France La Reunion		✓		✓
The Gambia		✓	✓	✓
Mali		✓	✓	✓
South Africa			✓	✓
Nigeria			✓	

Table 3-2: The burden of KS in sub-Saharan African countries in the regions over pre- and post- HIV/AIDS epidemic ART rollout periods (<1980, 1981-2000, 2001-2010 and 2011-2016).

sub-Saharan Africa regions / countries	Latest adult (15-49) HIV prevalence % (2018)[b]	Introduction of ART (Year)[c]	Latest estimated coverage of ART % (2018)[d]	Before 1980 KS ASIR per 100 000[e]		1981-2000 KS ASIR per 100 000[f]		2001-2010 KS ASIR per 100 000[g]		2011-2016 KS ASIR per 100 000[h,i]	
				Male	Female	Male	Female	Male	Female	Male	Female
Southern Africa[a]								**11.5**	**5.1**	**7.6**	**4.7**
Angola	2	2003	27			0.0	0.0				
Botswana	20.3	2002	83					24.3	15.4	19.1	9.9
Eswatini	27.3	2003	86			17.2	9.5			13.5	6.4
Lesotho	23.6	2004	61								
Madagascar	0.3	2003	9								
Namibia	11.8	2003	92			6.8	1.9	23.2	7.7	16.4	6.9
South Africa (Eastern Cape)	20.4	2004	62					5.8	3.6	6.0	3.5
Zambia	11.8	2002	78							14.8	8.1
Eastern Africa[a]								**14.9**	**6.8**	**15.1**	**7.6**
Burundi	1	2003	80								
Comoros	<0.1	2005	79								
Eritrea	0.7	2005	51								
Ethiopia	1	2005	65					0.6	0.3	0.6	0.5
Kenya (Eldoret)	4.7	2003	68			5.8	2.4	7.0	4.7	2.6	2.0
Kenya (Nairobi)								3.2	1.8	3.0	1.5
Malawi	9.2	2004	78	NC	NC	49.9	31.7	55.5	28.9		
Malawi (Blantyre)								91.8	43.6		
Mauritius	1.3	2001	22			0.0	0.0	0.0	0.0	0.0	0.0
Mozambique (Beira)	12.6	2004	56					63.6	27.5	32.0	11.2
Mozambique (Maputo)										22.7	11.6
France, La Reunion		2004				0.6	0.0			0.2	0.0
Rwanda	2.5	2003	87								

sub-Saharan Africa regions / countries	Latest adult (15-49) HIV prevalence % (2018)[b]	Introduction of ART (Year)[c]	Latest estimated coverage of ART % (2018)[d]	Before 1980 KS ASIR per 100 000[e]		1981-2000 KS ASIR per 100 000[f]		2001-2010 KS ASIR per 100 000[g]		2011-2016 KS ASIR per 100 000[h,i]	
				Male	Female	Male	Female	Male	Female	Male	Female
Seychelles		2001						0.0	0.0	0.0	0.0
Tanzania	4.6	2004	71							5.3	4.2
Uganda (Kampala)	N/A	2004	72	39.3	21.8	37.7	20.5	25.2	14.8	18.6	11.2
Uganda (Kyadondo)						37.9	20.4	29.5	20.2	24.0	14.1
Uganda (Gulu)										10.1	3.7
Zimbabwe (Bulawayo)	12.7	2004	88	2.6	0.3					15.5	6.0
Zimbabwe (Harare)						50.8	20.3	37.3	23.5	21.8	11.8
Central Africa[a]								**4.1**	**0.6**	**1.2**	**0.4**
Cameroon	3.6	2003	52								
Central African Republic	3.6	2003	36								
Chad	1.3	2006	51								
Congo (Democratic Republic)		2002	57								
Congo (Brazzaville)	2.6	2003	35			4.3	1.4	0.6	0.4	0.5	0.0
Equatorial Guinea	7.1	2005	34								
Gabon	3.8	2002	67								
Sao Tome & Principe	N/A	2005	N/A								
West Africa[a]								**1.9**	**1.2**	**0.9**	**0.6**
Benin	1	2002	61							0.4	0.0
Burkina Faso	0.7	2003	62								
Cape Verde	0.6	2004	89								
Cote d'Ivoire	2.6	2004	55			2.2	1.4			0.7	0.5
The Gambia	1.9	2004	29			0.6	0.4	0.3	0.1	0.7	0.2

sub-Saharan Africa regions / countries	Latest adult (15-49) HIV prevalence % (2018)[b]	Introduction of ART (Year)[c]	Latest estimated coverage of ART % (2018)[d]	Before 1980 KS ASIR per 100 000[e]		1981-2000 KS ASIR per 100 000[f]		2001-2010 KS ASIR per 100 000[g]		2011-2016 KS ASIR per 100 000[h,i]	
				Male	Female	Male	Female	Male	Female	Male	Female
Ghana	1.7	2004	34							0.8	0.2
Guinea	1.4	2003	40			0.2	0.0	0.9	0.3		
Guinea-Bissau	3.5	2005	33								
Liberia	1.3	2004	35								
Mali (Bamako)	1.4	2004	31	NC	NC	2.1	1.0	1.7	0.7	0.8	0.6
Mauritania	0.2	2003	54								
Niger	0.3	2005	54	NC	NC	0.3	0.2	0.1	0.1	0.4	0.1
Nigeria (Abuja)	1.5	2002	53							3.8	1.0
Nigeria (Calabar)										2.6	0.5
Nigeria (Ibadan)						0.2	0.1	0.4	0.3		
Senegal	0.4	1998	63	NC	NC						
Sierra Leone	1.5	2005	41								
Togo	2.3		60								

- [a]GLOBOCAN 2008 and 2012 reports (1,122).
- [b,c]UNAIDS/WHO epidemiological fact sheet 2004 update (123).
- [d]UNAIDS website and WHO website (85,86).
- [e,f,g,h]Population based cancer registries; AFCRN reports. Cancer in Africa Epidemiology and Prevention (1960-2000) (119). Cancer in Africa (2001-2014) (121), Cancer in sub-Saharan Africa AFCRN Vol III (120).
- [i]Population based cancer registries; CI5 reports. CI5 Vol VIII (1993-1998) (116). CI5 Vol X (2003-2007) (117). CI5 Vol XI (2008-2012) (118).

N/A: Not applicable.

NC: KS not classified.

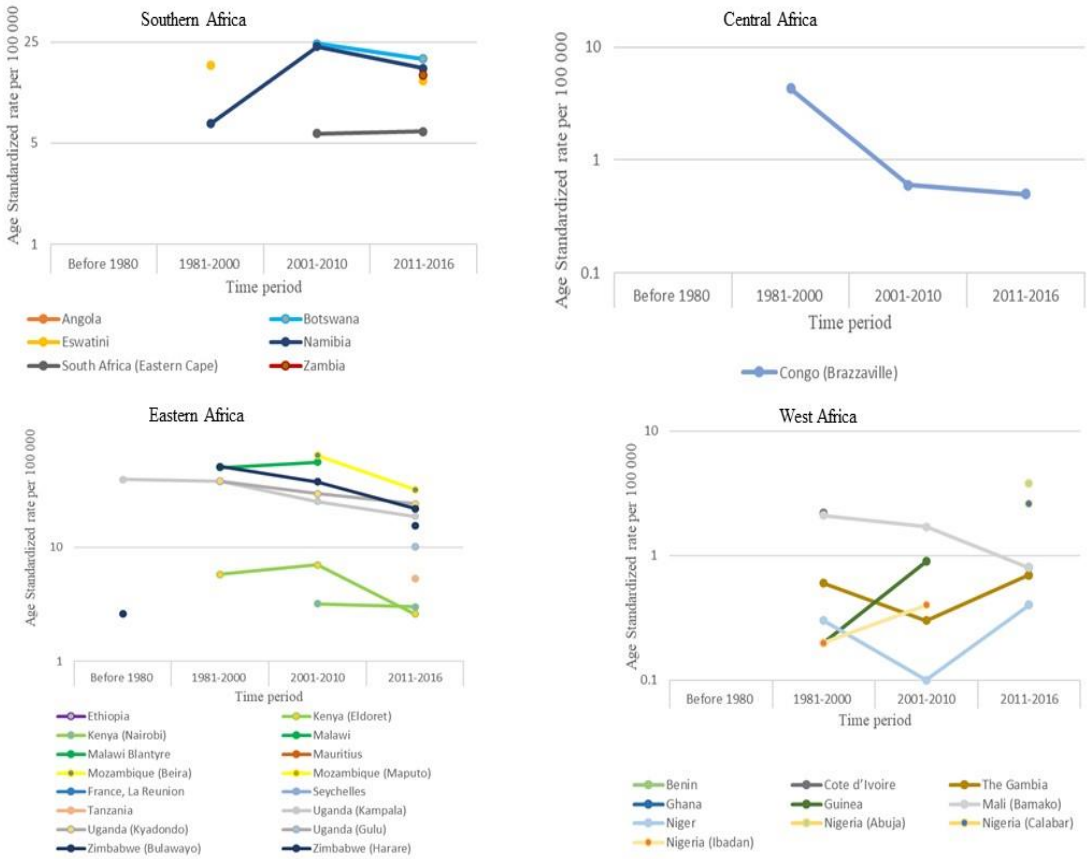

Figure 3-1: KS ASIR per 100,000 trends in males (before 1980 to 2016) in sub-Saharan African countries. Data source: Cancer in Africa Epidemiology and Prevention (1960-2000) (119). Cancer in Africa (2001-2014) (121). Cancer in sub-Saharan Africa AFCRN Vol III (120). Population based cancer registries. CI5 Vol VIII (1993-1998) (116). CI5 Vol X (2003-2007) (117). CI5 Vol XI (2008-2012) (118).

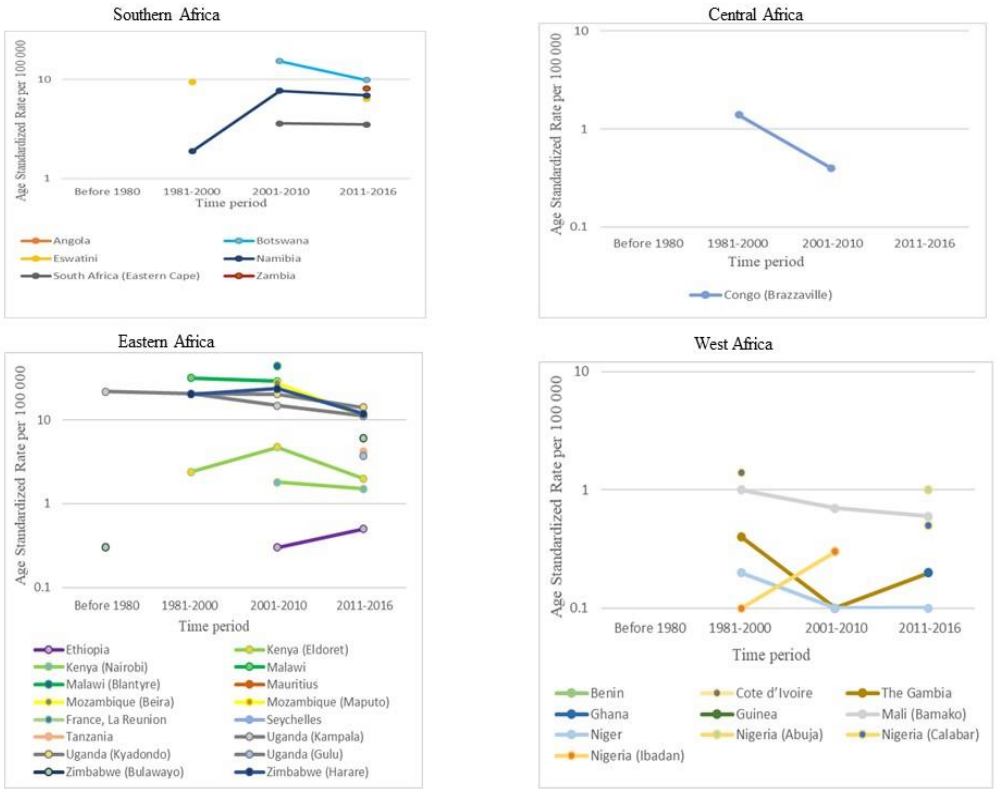

Figure 3-2: KS ASIR per 100,000 trends in females (before 1980 to 2016) from sub-Saharan African countries. Data source Cancer in Africa Epidemiology and Prevention (1960-2000) (119). Cancer in Africa (2001-2014) (121). Cancer in sub-Saharan Africa AFCRN Vol III (120). Population based cancer registries. CI5 Vol VIII (1993-1998) (116). CI5 Vol X (2003-2007) (117). CI5 Vol XI (2008-2012) (118).

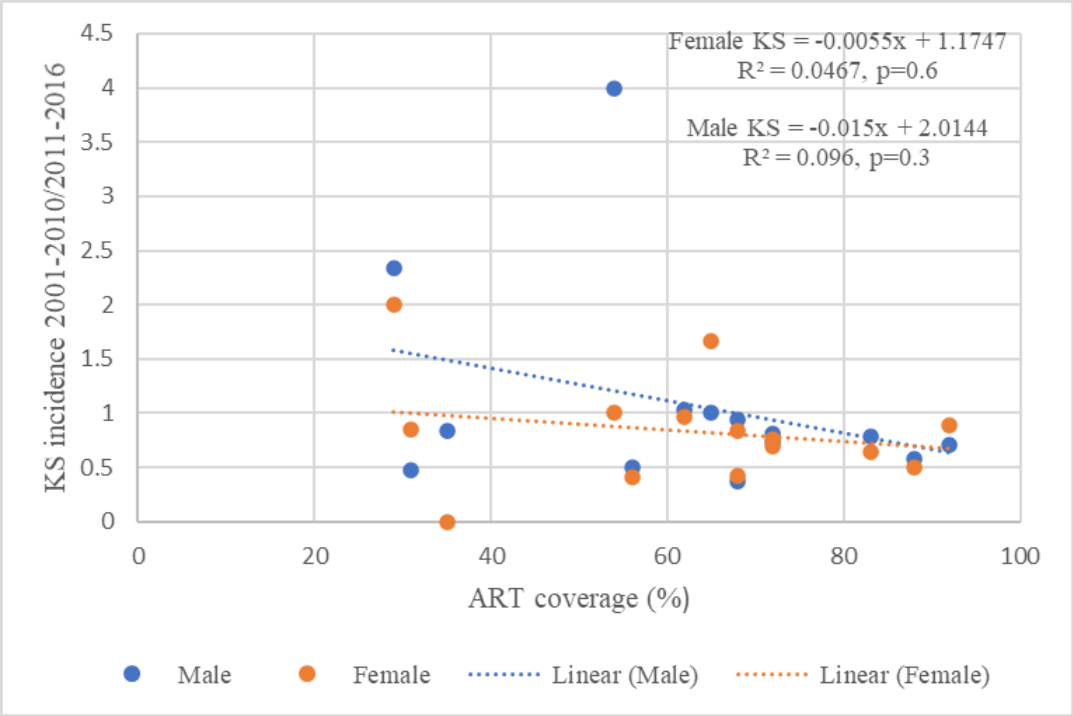

Figure 3-3: Relationship between ART coverage and decline in KS incidence between 2001 and 2010 (early ART coverage period) and 2011-2016 (fair to good ART coverage period). Data source: Cancer in Africa Epidemiology and Prevention (1960-2000) (119). Cancer in Africa (2001-2014) (121). Cancer in sub-Saharan Africa AFCRN Vol III (120). Population based cancer registries. CI5 Vol VIII (1993-1998) (116). CI5 Vol X (2003-2007) (117). CI5 Vol XI (2008-2012) (118).

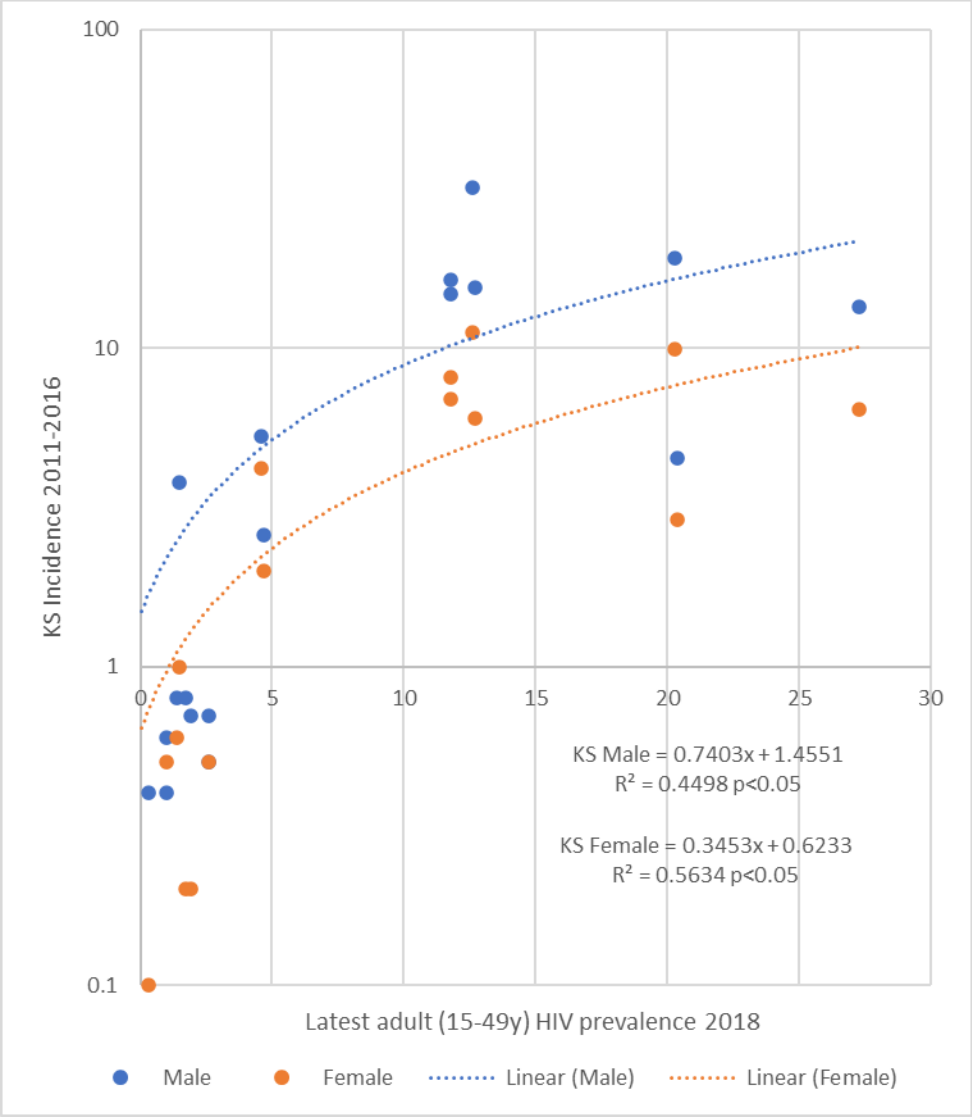

Figure 3-4: Relationship between latest adult (15-49) HIV prevalence and KS incidence between 2011 and 2016. Data source: Cancer in Africa Epidemiology and Prevention (1960-2000) (119). Cancer in Africa (2001-2014) (121). Cancer in sub-Saharan Africa AFCRN Vol III (120). Population based cancer registries. CI5 Vol VIII (1993-1998) (116). CI5 Vol X (2003-2007) (117). CI5 Vol XI (2008-2012) (118).

3.4 Discussion

3.4.1 Data quality and coverage

There is a significant under-reporting of cancer incidence in sub-Saharan Africa as there are few population-based cancer registries in the region. Cancer registration has been progressing slowly since the 1980s and this can be attributed to several factors like insufficient and poor coordination of data sources, lack of adequately trained staff, poor infrastructure and low to no political will in other countries (95,116,117). The KS incidence data used is from WHO-IARC and from AFCRN publications. These are considered to be reliable data source and cover about 24 countries – however not all of the data reported in the AFCRN are as strictly quality assured as in CI5. Prior to the onset of the HIV/AIDS epidemic in the 1980s there were clear geographical variations in KS incidence within Africa. These are not obvious from sparse, high quality incidence data but previous work using relative frequencies does illustrate wide variations between central & East Africa compared to areas north and south (124).

3.4.2. The effect of ART on KS incidence in sub-Saharan Africa

During periods 2001-2010 and 2011-2016, 11 out of 16 registries with sufficient trend data showed a decline in KS incidence. Changes in KS incidence between these two periods vary from a 63% decline (Kenya, males) to a 400% increase (Nigeria, males), a crude average decline of 6%. KS trend data from a similar period (2000-2016) from the United States of America show a decrease in incidence of 35% (125).

The decrease in KS incidence has been small, this was not associated with ART coverage as measured (28,95) and possibly more related to broader changes in the HIV prevalence in many

sub-Saharan African countries over the years. One study reported that between 2000 and 2017, the estimated country level HIV prevalence increased in 15 of the 46 sub-Saharan African countries, unfortunately changes in HIV prevalence are not necessarily linear or consistently in the same direction in the region (126). Another study reported a decline in HIV infections of more than 33% from an estimated 2.2 (2.1-2.3) million in 2005 to 1.5 (1.3-1.6) million in 2013, ART scale up and wide coverage were attributed to the substantial declines in new HIV infections (102).

Despite ART scale up in African countries HIV/AIDS-associated KS burden remains high in sub-Saharan Africa due to ART coverage gaps and policy variation (30,127). Total coverage and implementation of 'test and treat all' is still a challenge in many sub-Saharan African countries because of limited resources and logistical issues (30). In sub-Saharan Africa, the availability of ART has not completely resolved the challenges of epidemic KS (31). The availability of oncologic care services and the existing underlying comorbidities may also be contributing to the variations (94).

Some of the decline in KS incidence can be attributed to the introduction of ART in sub-Saharan Africa, in the early 2000s and a drop in the HIV prevalence over the years. This is substantiated by epidemiological studies in South Africa and Uganda that reported an increase in KS cases, as the HIV/AIDS epidemic progressed, followed by a subsequent decrease once ART was introduced (90,96,128). Epidemic KS is an important public health problem in sub-Saharan Africa because of the high KSHV and HIV prevalence (113–115). KSHV is common in sub-Saharan Africa countries like Cameroon and Uganda where KS was relatively frequent pre-1980, but the virus is also common in countries like Botswana and Gambia where KS was rare before the spread of HIV (7).

KS is more common in males. It is not clear why KS is much more frequent in males. This suggests the need for more studies on the cofactors in the etiology of KS (20), and ideally development of an effective KSHV vaccine.

3.5 Limitations

Only 2% of the continent's population was covered by cancer registries that complied with the IARC quality standards in 2014. This indicates significant lack of population-based registries in Africa (30,52). We used population-based registry data for cancer incidence, HIV prevalence and ART coverage data to reflect population trends. We acknowledge that the data are geographical, and the role of ART may not be ascertained using such correlation methods, differences in data sources used and large variations in ART rollout policies.

3.6 Conclusion

In sub-Saharan Africa, there is limited evidence of a decline in KS incidence after the rollout of ART in some countries. However, epidemic KS continues to be a major public health problem in terms of morbidity and mortality. There is a paucity of literature on the burden of KS, risk factors associated with KS and the impact of ART on KS. There is a need for direct measures of the incidence and risk factors for KS in sub-Saharan Africa and effects of treatments. These could then be used to inform interventions, prevention, and management policies.

The continued recording of high numbers of KS in sub-Saharan Africa contrasts with its decline in developed countries and could be attributed to the ART treatment gap in the sub-Saharan Africa. The long-term effect of ART on KS in resource-limited regions like sub-Saharan Africa still needs

to be evaluated to inform prevention and management interventions to improve the overall outcome for individuals living with HIV in this region. This requires continued investment in research of HIV associated malignancies in sub-Saharan Africa (30).

Chapter 4: Lifestyle factors associated with sex differences in Kaposi sarcoma incidence among adult black South Africans: A case-control study

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This chapter we assessed the prevalence of known cancer modifying factors (parity, hormonal contraceptive use in females, sex-partners, smoking and alcohol consumption in both sexes), and their relationship to KS, and whether any of these could account for the unequal KS sex ratios. KS has always been more common in adult males than females and there is a dearth of information on KS lifestyle risk factors that could explain these sex differences. Data for this paper was from the JCS. Cases for this study were participants with a histologically confirmed diagnosis of KS (N=1,275). Controls were women and men drawn from the rest (N=19,161) of the participants diagnosed with cancer types that have no known relationship to exposures of interest. The original paper publication has also been included in **Appendix 5**.

4.1 Introduction

KS is endemic in several countries in Southern and Eastern Africa and relatively rare cancer worldwide, with approximately 34,000 cases and 15,000 deaths reported by GLOBOCAN in 2020 (2). KS remains the most common cancer among PLWH (30,31). KS is classified as an AIDS-defining cancer and the risk of developing KS is about 50-134 fold higher among those individuals who are HIV positive in South Africa (27,28). Regardless of HIV positivity, throughout Africa, adult males have always been at higher risk of KS than females (23,32). In South Africa, between

1988 and 2017 the pathology-based National Cancer Registry reported that KS incidence rates doubled in males and increased about seven-fold in females. This resulted in a decline of the sex ratio from 7:1 (males versus females) in 1988 to 2:1 in 2017 (20,33,34). Even though the ratio has declined, most likely as a result of the nature of the HIV epidemic in South Africa which predominantly affects women, the KS sex disparities are still evident.

KSHV is considered a necessary cause of KS (9). Co-infection with HIV and KSHV greatly increases the likelihood of developing KS (19), which has resulted in a large increase in KS incidence during the HIV/AIDS pandemic in South Africa (34). In Africa, the seroprevalence of KSHV varies from 20-80% (19,21,27,33,57,60,115,129). A recent meta-analysis of KSHV prevalence showed no difference in KSHV seroprevalence between boys and girls in sub-Saharan Africa but found KSHV to be about 30% higher in adult males than females, which may explain some of the sex differences observed (32). In other studies conducted in Uganda, males were twice as likely to have detectable KSHV in blood compared to females (18,130,131), however in South Africa, we observed no Male: Female differences in adult KSHV seroprevalence (19). The more than two-fold KS sex differences suggest the importance of further unknown cofactors other than KSHV in the development of KS (20).

There is a dearth of information on KS lifestyle risk factors that could explain these sex differences (132). The role of immunity and genomic make-up is currently uncharted. Grulich and Kaldor in 1996, at the early stages of the HIV epidemic, suggested sex hormones (e.g. during pregnancy) were not an important factor in KS pathogenesis (133). Male hormones (androgen and testosterone) have been reported to aid KSHV infection and female pregnancy hormone human chorionic gonadotropin (hCG) inhibit the development of KS (134–138). The role of smoking as

a risk factor for KS was suggested by Goedert in 2009 to be protective in classical and epidemic KS in Sicily and the United States of America (139–141). The role of alcohol as a risk factor for KS is inconclusive (132). These studies were, all however, small.

With some exceptions, smoking and drinking prevalences are higher in men than in women. Males are more likely to be in the heavy drinking and smoking categories as compared to females in South Africa. Peltzer and colleagues reported 41.5% men compared to 17.1% women as current alcohol users from the 2008 South African national population survey, whereas for all ethnicities, hazardous/ harmful drinking in black males and females were 15.5% and 10.0% respectively (142). Results from the South African Smoking and Death Notification Survey (2007, aged 35-74) show black males had a smoking prevalence four times (46.3%) higher than black females (11.1%) (143).

We used data from the JCS to assess whether the lower incidence of KS in females (or higher incidence in males) could be attributed to any of known cancer-associated lifestyle factors such as parity, hormonal contraceptive use, lifetime sex partners, urban/rural residence, education, smoking and alcohol consumption in a black South African population.

4.2 Methods

4.2.1 Study setting and design

The JCS is an epidemiological study of over 25,000 cancer patients established by the National Cancer Registry of South Africa in 1995, at the early stages of the HIV epidemic. Data collection continued during the antiretroviral (ARV) rollout in 2004 and ended in 2016. The study aim of the

JCS described in Chen et al., (144) was to investigate the relative importance of known cancer risk factors in an African population. This study utilizes an established case-control design for cancer epidemiological studies, whereby cases are individuals with the cancer of interest and controls are individuals with other cancers that are not associated with the exposures under investigation (27,145,146).

4.2.2 Study population

The JCS recruited in a consecutive fashion, cancer patients of any type mainly from southern Gauteng province, attending the medical and radiation oncology departments of the largest tertiary referral public hospitals in Johannesburg (mainly Charlotte Maxeke hospital and associated referral centres) (144). Participants were aged 18 to 74 years, black, resident of South Africa, with a new (incident) diagnosis of cancer, who had given written or witnessed oral informed consent. Trained nurse interviewers used a standard questionnaire to interview participants in their preferred language (usually Zulu or Sotho). Participants were interviewed within six months of diagnosis and before receiving radio- or chemotherapy.

The questionnaire covered questions on the leading, emerging and suspected risk factors for cancer such as place of birth and residence, education, ethnicity (home language of parents), method of cooking and heating, smoking by type of tobacco and amounts smoked, snuff (sniffed tobacco) use, alcohol consumption by type and amount consumed, parity, use of oral and injectable contraceptives, number of sexual partners, basic occupations, and self-reported use of ART (since 2005). Interviewers collected peripheral blood samples for HIV testing and other analyses. The serum was used for serological screening for HIV on all samples and KSHV and Human papilloma

virus (HPV) in selected samples in previous studies (19,27). HIV antibody testing was conducted using the Vironostika (HIV Uniform II plus O) micro enzyme-linked immunosorbent assay (ELISA). Cancer types were mostly (>90% ascertained by histology (27) and coded to their topography and morphology using the ICD-O3.

4.2.3 Ethics

The University of Witwatersrand Human Research Ethics Committee (Medical) approved the primary study and the current study (Clearance certificate number: M191130).

4.2.4 Data management

Figure 4-1 shows the participants used in case-control selection. Cases for this study were participants with a histologically confirmed diagnosis of KS (N=1,275). Controls were women and men drawn from the rest (N=19,161) of the participants diagnosed with cancer types that have no known relationship to exposures of interest. We used the IARC 100th Monograph series and Schottenfeld and Fraumeni's Textbook (3rd and 4th editions), to assess cancer/exposure relationships to identify appropriate control cancer types (9,144,147–149). Control selection and therefore sample size varied when assessing the effect of HIV (infection-related cancers excluded, N=10,309 remained), smoking (smoking-related cancers excluded, N=2,170 remained), alcohol consumption (alcohol-related cancers excluded, N=3,090 remained), parity and hormonal contraceptive use (reproductive-related cancers were excluded, N=6,870 remained) (**Figure 4-1**). A detailed list of cancers included in each control group and the sex distribution among cases and controls (Supplementary **Table S4-1 and Table S4-2**). To test the robustness of the smoking and alcohol-KS-ORs we performed a sensitivity analysis recalculating these ORs by removing one

cancer type at a time from each of the control comparison groups. (Supplementary Figure **S4-1 and S4-2**).

The explanatory variables included sex, age group at enrolment, year of interview, place of birth (by province), ethnicity, place of residence (urban/rural), education (none, secondary or tertiary) and number of sexual partners (0-1, 2-5, 6 or more), weekly alcohol consumption (none, light (1-7 drinks for females and 1-14 drinks for males), and heavy (>8 drinks for females and >14 drinks for males). Cut-offs for heavy drinking were set at 8 or more drinks per week for women, 15 or more drinks per week for men according to the Centers for Disease Control and Prevention alcohol factsheet (150). A current smoker was defined as someone who stopped smoking 5 years before interview to minimize reverse causation, and we conservatively assumed 1 gram of tobacco per unit of tobacco products (mainly cigarettes). In females only, parity was classified as 0-2, 3 or more children born alive, and contraceptive use was measured in relation to combinations of ever/never use of injectable or oral contraceptives. Period of use of antiretroviral therapy period (ART-period) was classified as pre-ART (1995–2004), early ART (2005–2009) and late ART period (2010–2016).

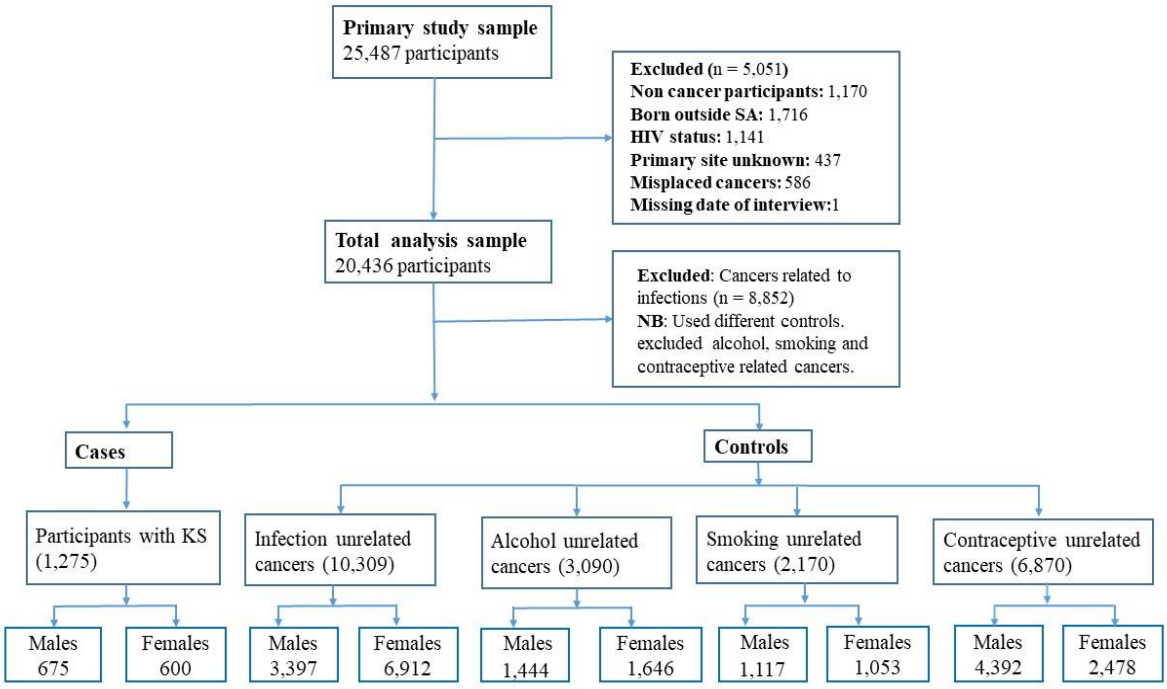

Figure 4-1: Selection of study participants (cases and controls).

4.2.5 Statistical analysis

The descriptive summary of the socio-demographic characteristics of cases and controls are shown in **Table 4-1**. To assess whether the high M:F sex ratio was associated with any of the leading modifying factors, unconditional unmatched logistic regression models were fitted to calculate their odds ratios (ORs) and 95% CI in relation to KS overall (**Table 4-2**) and stratified by sex (**Table4-3**). ORs were adjusted for important socio-demographic variables (sex-where appropriate, age group, year of interview, HIV status, number of sexual partners, education, place of residence and ART period). For alcohol consumption and smoking we assessed the effect of an interaction term between alcohol, smoking and sex using unmatched logistic regression models (Supplementary **Table S4-5 and S4-6**).

We performed a test for heterogeneity on categorical variables and a score test for trend in the ORs on ordinal categorical variables (**Table 4-2 and 4-3**). To adjust for confounding associated with HIV, smoking and alcohol consumption analyses were also restricted to HIV positive cases and controls. For each set of case-control comparisons, we used appropriate control comparison groups, conscious that the sample size in each set of comparisons varied. The statistical analysis was done using STATA software version 15.0 (Stata Corp, College Station, Texas).

4.3 Results

The majority of the 1,275 individuals diagnosed with KS were male, 675 (53.0%) (**Table 4-1**). Most of the KS cases were HIV positive (95.6% males and 96.7% females), with very little difference in HIV prevalence across all the demographic and lifestyle groups studied. The highest proportion of KS cases was between the ages of 25 and 34 years in females and 35 to 44 among males. The majority of the cases (95%) were from urban places of residence and had secondary/tertiary education level (72.4% males and 80% females). Males reported a median of six sexual partners (IQR=4–10) and females a median of four partners (IQR=3–5). Most males, (N=430, 63.7%) vs females (N=172, 28.7%) were in the heavy alcohol consumption category.

Of the 675 male KS cases, 13.6% (N=92) males vs 2.5% (N=15) of the 600 females smoked 15 grams or more of tobacco per day (current heavy smokers). Most females, 372 (62.0%) reported giving birth to 0–2 children. More than half of the females, 309 (51.5%) reported only using injectable contraceptives and 49 (8.17%) reported only using oral contraceptives. Among the controls, HIV prevalence was 11.2% in males and 17.9% in females. About three times as many males (35.6%) reported heavy alcohol consumption (8-15 or more drinks a week) vs. females

(11.6%). About five times as many males (40%) reported current smoking, vs. females (8%) (**Table 4-1**). Smoking and alcohol consumption prevalences are higher in males than females in both cases and controls. Socio-demographic characteristics of study participants by HIV status were also tabulated (Supplementary **Table S4-3**).

Table 4-1: Socio-demographic characteristics of study participants (cases and controls)

Characteristics	Cases (1,275)		Controls (10,309[a])	
	Males	Females	Males	Females
	Total 675 (52.94) N (Col %)	Total 600 (47.06) N (Col %)	Total 3,397 (32.95) N (Col %)	Total 6,912 (67.05) N (Col %)
HIV status				
Negative	30 (4.44)	20(3.33)	3,018 (88.84)	5,674 (82.09)
Positive	645 (95.56)	580 (96.67)	379 (11.16)	1,238 (17.91)
Age				
Median [IQR]*	38 [33 – 44]	33 [29 – 40]	57 [49 – 64]	52 [43 – 61]
Age group (years)				
18-24	11 (1.63)	54 (9.00)	79 (2.33)	84 (1.22)
25-34	214 (31.70)	282 (47.00)	122 (3.59)	503 (7.28)
35-44	292 (43.26)	169 (28.17)	324 (9.54)	1,395 (20.18)
45-54	120 (17.78)	69 (11.50)	887 (26.11)	1,921 (27.79)
55-64	30 (4.44)	24 (4.00)	1,197 (35.24)	1,864 (26.97)
65+	8 (1.19)	2 (0.33)	788 (23.20)	1,145 (16.57)
Period of interview				
1995-1999	41 (6.07)	33 (5.50)	827 (24.35)	1,057 (15.29)
2000-2004	121 (17.93)	113 (18.83)	673 (19.81)	1,217 (17.61)
2005-2009	229 (33.93)	229 (38.17)	895 (26.35)	1,848 (26.74)
2010-2016	284 (42.07)	225 (37.50)	1,002 (29.50)	2,790 (40.36)
Place of residence				
Urban	647 (95.85)	564 (94.00)	3,020 (88.90)	6,187 (89.51)
Rural	26 (3.85)	34 (5.67)	362 (10.66)	697 (10.08)
Missing	2 (0.30)	2 (0.33)	15 (0.44)	28 (0.41)
Education				
None	31 (4.59)	24 (4.00)	462 (13.60)	740 (10.71)
Primary	155 (22.96)	96 (16.00)	1,330 (39.15)	2,023 (29.27)
Secondary & Tertiary	489 (72.44)	480 (80.00)	1,596 (46.98)	4,130 (59.75)
Missing	-	-	9 (0.26)	19 (0.27)
Number of sexual partners				
Median [IQR]	6 [4 – 10]	4 [3 – 5]	5 [3 – 9]	3 [2 – 5]
Number of sexual partners (cat)				
0-1	16 (2.37)	24 (4.00)	153 (4.50)	655 (9.48)
2-5	207 (30.67)	333 (55.50)	1,319 (38.83)	3,860 (55.84)
6 or more	265 (39.26)	115 (19.17)	1,176 (34.62)	674 (9.75)

Characteristics	Cases (1,275)		Controls (10,309[a])	
	Males	Females	Males	Females
	Total 675 (52.94) N (Col %)	Total 600 (47.06) N (Col %)	Total 3,397 (32.95) N (Col %)	Total 6,912 (67.05) N (Col %)
Unknown	187 (27.70)	128 (21.33)	749 (22.05)	1,723 (24.93)
Alcohol consumption[b]				
Never	203 (30.07)	382 (63.67)	845 (58.55)	1,368 (83.11)
Light >0-7 or >14 drinks/week	42 (6.22)	46 (7.67)	85 (5.88)	87 (5.29)
Heavy ≥8 or ≥15 drinks/week	430 (63.70)	172 (28.67)	514 (35.57)	191 (11.60)
Smoking[c]				
Never	206 (30.52)	483 (80.50)	393 (35.18)	891 (84.62)
Ex-smoker	69 (10.22)	20 (3.33)	273 (24.44)	71 (6.74)
Current-light 1-14g/day	308 (45.63)	82 (13.67)	348 (31.15)	83 (7.88)
Current-heavy ≥15g/day	92 (13.63)	15 (2.50)	103 (9.22)	6 (0.57)
Missing	-	-	-	2 (0.19)
Parity[d]				
0-2		372 (62.00)		866 (34.94)
3 or more		153 (25.50)		1,436 (57.95)
Unknown		75 (12.50)		176 (7.10)
Contraceptive use[d]				
Never oral (oc) or injectable (ic)		152 (25.33)		1,318 (53.19)
Ever oc never ic		49 (8.17)		271 (10.94)
Ever ic never oc		309 (51.50)		543 (21.91)
Ever ic and ever oc		88 (14.67)		332 (13.40)
#Ever ic and/ or ever oc		446 (74.33)		1,146 (46.25)
Missing		2 (0.33)		14 (0.56)
ART-period				
Pre-ART 1995-2004	162 (24.00)	146 (24.33)	1,500 (44.16)	2,274 (32.90)
Early ART 2005-2009	229 (33.93)	229 (38.17)	895 (26.35)	1,848 (26.74)
Late ART 2010-2016	284 (42.07)	225 (37.50)	1,002 (29.50)	2,790 (40.36)

Abbreviations: IQR, Interquartile range; oc, oral contraceptive; ic, injectable contraceptive. * Median age [IQR].
#Ever ic and /or ever oc: a sum of category (2 to 4), ever oc never ic, ever ic never oc and ever ic and ever oc.
Total number of controls for the lifestyle exposures are different: for the demographic variables, infection unrelated cancer controls were used. For alcohol consumption, the controls comprised participants with cancers that were unrelated to infectious agents and alcohol. For smoking, the controls were not related to infectious agents and smoking. Contraceptive use controls were cancers unrelated to infectious agents and hormonal contraceptives.

[a]Infection unrelated controls=10,309 [b]Alcohol unrelated controls=3,090 [c]Smoking unrelated controls=2,170 [d]Contraceptive unrelated controls=6,870

Table 4-2: Unadjusted and adjusted ORs (95% CI) of developing KS

Variables	Unadjusted OR (95% CI) using appropriate controls	Adjusted OR (95% CI) using appropriate controls
HIV status		
Negative	1.00	1.00
Positive	131.70 (98.77 – 175.60)	**115.42 (79.76 – 167.01)**
P-value (heterogeneity)	<0.001	<0.001
Place of residence		
Urban	1.00	1.00
Rural	0.43 (0.33 – 0.56)	0.83 (0.54 – 1.28)
P-value (heterogeneity)	<0.001	0.181
Education		
None	1.00	1.00
Primary	1.64 (1.21 – 2.21)	0.86 (0.56 – 1.32)
Secondary & Tertiary	3.70 (2.80 – 4.89)	0.86 (0.57 – 1.31)
P-value (heterogeneity)	<0.001	0.876
Number of sexual partners		
0-1	1.00	1.00
2-5	2.11 (1.51 – 2.93)	1.26 (0.77 – 2.08**)**
6 or more	4.15 (2.96 – 5.81)	1.32 (0.79 – 2.21)
Unknown	2.57 (1.83 – 3.61)	1.24 (0.73 – 2.11)
P-value (trend)	<0.001	0.402
Alcohol consumption[b]		
Never	1.00	1.00
Light	1.94 (1.47 – 2.54)	1.33 (0.87 – 2.04)
Heavy	3.23 (2.80 – 3.72)	**1.31 (1.03 – 1.67)**
P-value (trend)	<0.001	<0.001
P-value (sex contribution)		<0.001
Alcohol consumption[b] (in HIV positives only)		
Never	1.00	1.00
Light	1.38 (0.91 – 2.11)	1.32 (0.83 – 2.09)
Heavy	1.86 (1.50 – 2.32)	**1.35 (1.04 – 1.76)**
P-value (trend)	<0.001	<0.001
Smoking[c]		
Never	1.00	1.00
Ex-smoker	0.48 (0.38 – 0.62)	1.00 (0.65 – 1.55)
Current-light (1-14g)	1.69 (1.43 – 1.99)	**1.41 (1.03 – 1.92)**
Current-heavy (>=15g)	1.83 (1.38 – 2.43)	**1.82 (1.07 – 3.10)**
P-value (trend)	<0.001	<0.001
P-value (sex contribution)		0.013
Smoking[c] (in HIV positives only)		
Never	1.00	1.00
Ex-smoker	0.79 (0.52 – 1.18)	1.11 (0.68 – 1.81)
Current-light (1-14g)	1.70 (1.28 – 2.26)	1.26 (0.90 – 1.77)
Current-heavy (>=15g)	2.47 (1.41 – 4.34)	1.84 (0.99 – 3.40)
P-value (trend)	<0.001	0.087
Parity[d] (females only)		
0-2	1.00	1.00
3 or more	0.25 (0.20 – 0.30)	0.80 (0.59 – 1.09)
Unknown	1.00 (0.74 – 1.33)	0.83 (0.41 – 1.30)

Variables	Unadjusted OR (95% CI) using appropriate controls	Adjusted OR (95% CI) using appropriate controls
P-value (trend)	0.9578	0.4590
Contraceptive use[d] (females only)		
Never oral or injectable	1.00	1.00
Ever oc never ic	1.57 (1.11 – 2.22)	1.48 (0.89 – 2.47)
Ever ic never oc	4.93 (3.97 – 6.14)	1.19 (0.86 – 1.64)
Ever ic and ever oc	2.30 (1.72 – 3.07)	1.25 (0.81 – 1.94)
Ever ic and/ or ever oc	3.37 (2.76 – 4.12)	1.24 (0.91 – 1.68)
P-value (trend)	<0.001	0.3575
ART-period		
Pre-ART 1995-2004	1.00	1.00
Early ART 2005-2009	2.05 (1.76 – 2.38)	1.15 (0.89 – 1.48)
Late ART 2010-2016	1.64 (1.42 – 1.91)	0.83 (0.64 – 1.09)
P-value (trend)	<0.001	0.175

Demographic characteristics adjusted for sex, HIV status, age group, year of interview, place of birth, ethnicity, place of residence, education, number of sexual partners and ART period using infection unrelated controls[a]. [b]Alcohol comparisons used alcohol unrelated controls and adjusted for sex, age group, year of interview, education HIV status, number of sexual partners, place of residence and ART period. [c]Smoking comparisons used smoking unrelated controls and adjusted for sex, age group, year of interview, HIV status, education, number of sexual partners place of residence and ART period. [d]Contraceptive use and parity analysis for females only using reproductive factor unrelated controls, adjusted for age group, year of interview, HIV status, education, place of residence, number of sexual partners and ART period. Statistically significant OR_{adj} (95%CI) bolded.

KS risk was highest among persons who tested HIV positive, OR_{adj} of 115.42 (95% CI=79.76–167.01). We found no association between KS and number of sexual partners (P_{trend}=0.40). Persons in the heavy drinking category were more likely to develop KS with an OR_{adj} of 1.31 (95% CI=1.03–1.67) compared to never drinking category (P_{trend}<0.001). We found a strong statistical effect of sex in the overall model (Table 2, P_{value}<0.001).

Additional analyses

After restricting the analysis to persons who were HIV positive (remaining N=1,225 KS, 1,617 controls- i.e. losing approx. 80% of the controls) the odds of developing KS remained unchanged (OR_{adj}=1.35 (95%CI=1.04–1.76, P_{trend} <0.001)) in the heavy drinking category (**Table 4-2**). Those in the current heavy smoking category were more likely to develop KS (OR_{adj} 1.82 (95% CI=1.07–

3.10, P_{trend}<0.001) compared to never smoking (**Table 4-2**). We found a strong statistical effect of sex in the overall model (Table 2, P_{value}<0.013). Stratification of the data by sex is shown in **Table 4-3**. We found no evidence of sex*smoking (P-value 0.7) or sex*alcohol interactions (P-value=0.8) (Supplementary **Table S4-5 and S5-6**). We also tested the robustness of the smoking and alcohol KS associations by removing sex specific cancers from the analysis. We found no material differences in the ORs before and after removal (results not shown).

Table 4-3: Unadjusted and adjusted OR (95%CI) of developing KS stratified by sex

Variables	Unadjusted OR (95% CI)		Adjusted OR (95%CI)	
	Males	**Females**	**Males**	**Females**
HIV status[a]				
Negative	1.00	1.00	1.00	1.00
Positive	**171.21 (116.93 – 250.68)**	**132.91 (84.75 – 208.44)**	**116.70 (71.35 – 190.88)**	**93.91 (54.22 – 162.64)**
P-value (heterogeneity)	<0.001	<0.001	<0.001	<0.001
Alcohol consumption[b]				
Never	1.00	1.00	1.00	1.00
Light drinking	**2.06 (1.38 – 3.07)**	**1.89 (1.30 – 2.75)**	1.29 (0.68 – 2.46)	1.41 (0.79 – 2.50)
Heavy drinking	**3.48 (2.85 – 4.25)**	**3.22 (2.55 – 4.08)**	**1.46 (1.03 – 2.06)**	1.21 (0.85 – 1.72)
P-value (trend)	<0.001	<0.001	0.070	0.148
Alcohol consumption[b] (HIV positives)				
Never	1.00	1.00	1.00	1.00
Light drinking	1.00 (0.54 – 1.85)	1.73 (0.96 – 3.14)	1.08 (0.54 – 2.16)	1.63 (0.86 – 3.07)
Heavy drinking	**1.84 (1.33 – 2.57**)	**1.48 (1.07 – 2.05)**	**1.52 (1.04 – 2.23)**	1.21 (0.84 – 1.76)
P-value (trend)	<0.001	0.012	0.034	0.100
Smoking[c]				
Never	1.00	1.00	1.00	1.00
Ex-smoker	**0.48 (0.35 – 0.66)**	**0.52 (0.31 – 0.86)**	0.87 (0.52 – 1.47)	1.49 (0.64 – 3.45)
Current-light	**1.69 (1.34 – 2.12)**	**1.82 (1.32 – 2.52)**	**1.51 (1.01 – 2.26)**	1.21 (0.72 – 2.04)
Current-heavy	**1.70 (1.23 – 2.37)**	**4.61 (1.78 – 11.96)**	**1.83 (1.00 – 3.35)**	2.03 (0.51 – 8.12)
P-value (trend)	<0.001	<0.001	0.010	0.621
Smoking[c] (HIV positives)				
Never	1.00	1.00	1.00	1.00
Ex-smoker	0.73 (0.44 – 1.24)	0.68 (0.31 – 1.49)	1.19 (0.65 – 2.17)	1.13 (0.46 – 2.76)
Current-light	**1.73 (1.15 – 2.61)**	1.17 (0.72 – 1.90)	1.48 (0.94 – 2.31)	0.97 (0.57 – 1.65)
Current-heavy	**2.14 (1.12 – 4.10)**	2.81 (0.64 – 12.43)	1.98 (0.98 – 3.99)	2.43 (0.52 – 11.26)
P-value (trend)	<0.001	0.283	0.077	0.630

[a]For males and females, demographic characteristics used infection unrelated controls and adjusted for HIV status, age group, year of interview place of birth, ethnicity, place of residence, education, number of sexual partners and ART period. **[b]**Alcohol using alcohol unrelated controls and adjusted for sex, age group, year of interview, education, HIV status, place of residence, number of sexual partners and ART period. **[c]**Smoking using smoking unrelated controls and adjusted for sex, age group, year of interview, education, HIV status, place of residence, number of sexual partners and ART period.

HIV positive males were similarly likely to develop KS as compared to HIV infected females (OR_{adj}=116.70; 95%CI=71.35–190.88 versus OR_{adj}=93.91; 95%CI=54.22–162.40) (**Table 4-3**). The association between smoking and KS remained positive in males for both smoking (P_{trend}=0.034) and alcohol consumption (p-trend=0.01). In females, elevated ORs were observed in the heavy drinking and smoking categories (OR=1.21 and 2.03) but the p-trend is no longer significant (P_{trend}=0.1, 0.6 respectively) perhaps because of reduced sample sizes.

4.4 Discussion

We attempted to identify potential lifestyle explanations for the well described excess male to female sex ration in KS. We found that as in previous study (27) being HIV positive increased significantly the risk of KS development. In contrast to some previous studies (see below) we found an association between increased smoking intensity and KS and heavy alcohol consumption and KS particularly in males. Smoking and alcohol consumption may provide a possible explanation for the KS sex differences. We found no association between KS and parity or hormonal contraceptive use, reconfirming the hypothesis stated by Grulich and Kaldor (133). In a study estimating the prevalence of KSHV in females, de Sanjose and colleagues also reported that KSHV was not associated with the number of children, or patterns of oral contraceptive use (151). Additional studies of the potential mechanisms for sex-related differences in KS are necessary to evaluate further the roles of sex-specific factors such as parity and female sex hormones in KS pathogenesis (152).

As regards smoking, we found an association between increasing smoking intensity and KS, (**Table 4-3**). The association was evident in both sexes and in males alone, with sample sizes in

females becoming being too low to draw clear inferences. The literature regarding the role of smoking is mixed. An inverse association between cigarette smoking and infection with KSHV or KS development has been reported in some studies, but these results were not adjusted for education and other confounding factors (140,141,153). In one study in Uganda (N=458 KS cases) and another in Cameroon (N=266 cases), smoking was positively associated with HIV-related-KS; both studies adjusted for education and occupation associated with affluence (154,155). Our study based on over 1,000 KS cases and after adjustment for a range of confounders including education, does support the hypothesis that smoking increases the risk of developing KS, and also given male smoker rates are about five times those of females, this could provide a possible explanation for the KS sex differences. Larger sample size studies, meta- analyses or cohort studies are needed to confirm this hypothesis as smoking makes a partial contribution to the KS sex difference.

While the initial analysis of the association between KS and alcohol is interesting, a crude 3-fold OR among those (in both sexes combined) in the heavy drinking category was reduced to 1.31 after adjustment, so significant confounding appears to be involved (**Table 4-2**). After stratifying by sex, those in the heavy drinking category (vs. non-drinking) were associated with increased KS risk in males but the association became weaker in females (**Table 4-3**). Restricting the analysis further to HIV positive participants indicated that heavy alcohol consumption was still associated with increased odds of KS in males and the effect being less important in females (although this could be due to reduced sample size). In this study, it seems plausible that alcohol consumption is another possible explanation for the KS sex ratios, especially given alcohol consumption is three times more prevalent in males. The relationship between KS and alcohol consumption is complex as the behavioral patterns associated with alcohol consumption may influence the probability of

exposure to KSHV as stated by Mbulaiteye et al (156). In a case-control study of KS in HIV positive Ugandan patients, Ziegler et al. and Nansseu et al. found no associated risk between alcohol consumption and KS (154,155). KSHV seropositivity was also not associated with alcohol consumption in the study of Ugandan HIV-negative KS patients (156,157).

Other explanations for the KS sex differences include genetic make-up along with unmeasured hormonal differences (158) and immune system functions (159). Androgens aid KSHV infection and this may be why KS is more common in males than females (138). Androgen receptor (found in both males and females) expression in males is high and has been identified as a KSHV entry factor and subsequently promotes KSHV infection in cells (134,135). In females, the pregnancy hormone, human chorionic gonadotropin (hCG) has been reported to have shown anti-KS properties and this results in the reduction of KS tumor growth and inhibition of growth of other angiogenic tumors (137). This hormone has also been shown to inhibit the growth of KS cell lines in vitro (136). However, to the extent that we could measure (parity, hormonal contraception) we found no association between those factors in females and KS risk.

4.5 Limitations

Our study must be considered in light of possible limitations inherent in case-control studies and in this situation using people with a range of other cancers as controls. Cancer incidence in the South African black population is higher in females (National Cancer Registry data for 2017: 18,814 vs 13,116) (34) and thus they are also more frequently represented in the JCS (7,512 vs 4,072). This unusual situation, dominated by cancer of the cervix, distorts odds ratio calculations of M:F KS differences. In the analysis of risk factors, however, careful selection of controls

unrelated to exposures of interest allows for valid comparisons. Population prevalence estimates for smoking and alcohol, or reproductive factors that are comparable with the 18-74-year-old black, urban population comprising the JCS which collected data over a 20-year period, situated in southern Gauteng province are sparse. With that limitation in mind, national surveys (142) show prevalences of alcohol drinking of 35% in males and 10% in females with higher urban prevalences (in keeping with our observed prevalence of 41% and 17% in our mainly urban controls). Similarly, national current smoking prevalence in South African blacks is 50% in males and 12% in females (143), in keeping with our observed 56% and 8% prevalence in our controls, HIV prevalence in Gauteng province is 14% (150), again comparable to what is observed in our controls (11% in males 18% in females).

Recall of past exposures such as number of sexual partners, alcohol consumption and smoking could be subject to recall or desirability bias. JCS collected data from radiotherapy and oncology departments and therefore, cases that were not referred to these departments (such as surgery only or palliative care) were missed. There may be possible residual confounding of lifestyle factors and HIV. Since 97% of all KS patients were HIV positive, there may be possible associations with high-risk behaviors such as number of sexual partners, smoking and alcohol drinking which are known to be associated with HIV, and the age pattern (reproductive age group). We accounted for HIV confounding by restricting the analysis for smoking and alcohol consumption to those who were HIV positive. Despite these limitations, these data remain an important source to understand sex disparities in KS among the adult black South African population.

4.6 Strengths

Both cases and controls are people with incident cancers, thus interviewer and recall bias is minimized (and is non-differential) by interviewing cases and controls with diseases of similar severity. Referral biases are also minimized because both cases and controls are likely to be come from the same catchment areas and referred through the same process and departments (as a precaution we adjust for urban/rural residence). Over 95% of the cancers were histologically verified (27). Controls for this analysis were carefully selected, comprising cancer types not known to be associated with the exposures of interest (infectious agents, alcohol consumption smoking and hormonal contraceptive use). Sensitivity analysis of the controls used (Supplement **Figure S4-1 and S4-2**) shows control selection was robust.

4.7 Conclusion

We have assessed, in a systematic fashion, whether selected lifestyles that are known to cause cancer are associated with KS development and whether these can explain the greater male to female KS ratio. In this study, being HIV positive increased the risk of KS and the risk was higher among females compared to males. Parity and hormonal contraception use were not associated with KS among females. We found increased risks of KS (in both sexes combined) in relation to smoking and alcohol, and these risks remained positive after sex stratification and restriction to HIV positive participants. While confounding and biases cannot be fully ruled out, tobacco smoking and alcohol are therefore possibly responsible for the uneven KS sex ratios, given drinking and smoking was three to five times more common in males. While this is the largest case-control study to investigate these factors, reductions in sample size by restricting to HIV positives and by sex reduced our precision but the OR estimates remained about the same. Further

comparative research, preferably from large cohort studies is needed to elucidate whether smoking and alcohol act independently or whether they modify the role of HIV and KSHV, the two viruses responsible for KS, especially their viral loads, immunological responses and in identifying other genetic differences in the way the two sexes respond to HIV/KSHV infection.

Table S4-1: List of cancers included in each control group

Cancer type	**ICD-O-3-code**	**Frequency N**	**Infection unrelated cancer controls N=10,309**	**Smoking unrelated cancer controls N=2,170**	**Alcohol unrelated cancer controls N=3,090**	**Hormonal contraceptive unrelated cancer controls N= 6,870**
Breast	C50	4,479	✓			
Colon	C18-20	831	✓			✓
Endometrium	C54-55	392	✓	✓	✓	
Lung Cancer	C33-34	915	✓			✓
Myeloid Leukaemia	ICD-10 C92	275	✓		✓	✓
Myeloma	C90	307	✓	✓	✓	✓
Oesophagus	C15	1,234	✓			✓
Ovaries	C56	324	✓		✓	
Pancreas	C25	178	✓			✓
Peripheral nerves and ANS	C47	1	✓	✓	✓	✓
Melanoma	C43	82	✓	✓	✓	✓
Larynx	C32	342	✓			✓
Kidney	C64	61	✓			✓
Fallopian tube	C57.0	37	✓	✓	✓	
Meninges	C70	3	✓	✓	✓	✓
Endocrine gland	C75	6	✓	✓	✓	✓
Bone	C40-41	132	✓	✓	✓	✓
Brain	C71	41	✓	✓	✓	✓
Central nervous system (CNS)	C72	2	✓	✓	✓	✓
Peritoneum and retroperitoneum	C48	13	✓	✓	✓	✓
Placenta	C58.9	40	✓	✓	✓	
Prostrate	C61	499	✓	✓	✓	
Scrotum	C63.2	6	✓	✓	✓	
Small Intestine	C17	21	✓	✓	✓	✓
Testes	C62	27	✓	✓	✓	
Thymus	C37	9	✓	✓	✓	✓
Thyroid	C73	52	✓	✓	✓	
Eye and Adnexa	C69	85		✓	✓	✓
Soft tissue sarcoma	C49	232		✓	✓	✓

Cancer type	ICD-O-3-code	Frequency N	Infection unrelated cancer controls N=10,309	Smoking unrelated cancer controls N=2,170	Alcohol unrelated cancer controls N=3,090	Hormonal contraceptive unrelated cancer controls N= 6,870
Squamous cell carcinoma	C44	183		✓	✓	✓
Anus	C21	110			✓	✓
Leukaemia (not Myeloid)	C91-95	170			✓	✓
Penis	C60	41			✓	
Bladder	C67	89				✓
Nasal cavity and nasopharynx	C11, C30, C31	198				✓
Oral cavity and pharynx	C00-10, C12-14	998				✓
Stomach	C16	352				✓

Table S4-2: Sex distribution of the cases and the controls

Variable	Total N	Males N (%)	Females N (%)
Cases			
Kaposi sarcoma	1,275	675 (52.94)	600 (47.06)
Controls			
Infection unrelated	10,309	3,397 (32.95)	6,912 (67.05)
Alcohol unrelated	3,090	1,444 (46.73)	1,646 (53.27)
Smoking unrelated	2,170	1,117 (51.47)	1,053 (48.53)
Contraceptive use unrelated	6,870	4,392 (63.93)	2,478 (36.07)

Table S4-3: Socio-demographic characteristics of study participants (cases and controls) by HIV status

Characteristics	Cases (1,275)				Controls (10,309*)			
	Males		Females		Males		Females	
	Total 675 (52.94) N	HIV Positive=645 (95.7) N (%)	Total 600 (47.06) N	HIV Positive=580 (96.7) N (%)	Total 3,397 (32.95) N	HIV Positive= 379 (11.2) N (%)	Total 6,912 (67.05) N	HIV Positive= 1,238 (17.9) N (%)
Age								
Median (IQR)	38 (33 – 44)	38 (33 – 44)	33 (29 – 40)	33 (29 – 40)	57 (49 – 64)	51 (44 – 58)	52 (43 – 61)	44 (37 – 53)
Age group (years)								
18-24	11	11 (100)	54	53 (98.2)	79	3 (3.8)	84	13 (15.5)
25-34	214	208 (97.2)	282	274 (97.2)	122	23 (18.9)	503	201 (40.0)
35-44	292	285 (97.6)	169	167 (98.8)	324	75 (23.2)	1 395	407 (29.2)
45-54	120	114 (95.0)	69	62 (89.9)	887	137 (15.5)	1 921	355 (18.5)
55-64	30	25 (83.3)	24	23 (95.8)	1,197	100 (8.4)	1 864	202 (10.8)
65+	8	2 (25.0)	2	1 (50.0)	788	41 (5.2)	1 145	60 (5.2)
Period of interview								
1995-1999	41	31 (4.81)	33	27 (4.66)	827	39 (10.29)	1,057	68 (5.49)
2000-2004	121	113 (17.52)	113	111 (19.14)	673	60 (15.83)	1,217	152 (12.28)
2005-2009	229	227 (35.19)	229	226 (38.97)	895	121 (31.93)	1,848	334 (26.98)
2010-2016	284	274 (42.48)	225	216 (37.24)	1,002	159 (41.95)	2,790	684 (55.25)
Place of residence								
Urban	647	622 (96.1)	564	546 (96.8)	3,020	342 (11.3)	6,187	1,166 (18.9)
Rural	26	21 (80.8)	34	32 (94.1)	362	34 (9.4)	697	70 (10.0)
Missing	2	2 (100)	2	2 (100)	15	3 (20.0)	28	2 (7.1)
Education								
None	31	31 (100)	24	22 (91.7)	462	42 (9.1)	740	87 (11.8)
Primary	155	140 (90.3)	96	93 (96.9)	1,330	150 (11.3)	2,023	301 (14.9)
Secondary & Tertiary	489	474 (96.9)	480	465 (96.9)	1,596	187 (11.7)	4,130	847 (20.5)
Missing	-	-	-	-	9	0 (0.0)	19	3 (15.8)
Number of sexual partners								
Median (IQR)	6 (4 – 10)	6 (4 – 10)	4 (3 – 5)	4 (3 – 5)	5 (3 – 9)	6 (4 – 10)	3 (2 – 5)	4 (3 – 5)
Number of sexual partners (cat)								
0-1	16	14 (87.5)	24	21 (87.5)	153	11 (7.2)	655	43 (6.6)
2-5	207	200 (96.6)	333	327 (98.2)	1,319	110 (8.3)	3,860	626 (16.2)
6 or more	265	252 (95.1)	115	111 (96.5)	1,176	154 (13.1)	674	167 (24.8)
Unknown	187	179 (95.7)	128	121 (94.5)	749	104 (13.9)	1,723	402 (23.3)
Alcohol consumption**								
Never	203	186 (91.6)	382	365 (95.6)	845	88 (10.4)	1,368	220 (16.1)
Light >0-7 or >14 drinks/w	42	38 (90.5)	46	46 (100)	85	18 (21.2)	87	16 (18.4)

Characteristics	Cases (1,275)				Controls (10,309*)			
	Males		Females		Males		Females	
	Total 675 (52.94) N	HIV Positive=645 (95.7) N (%)	Total 600 (47.06) N	HIV Positive=580 (96.7) N (%)	Total 3,397 (32.95) N	HIV Positive= 379 (11.2) N (%)	Total 6,912 (67.05) N	HIV Positive= 1,238 (17.9) N (%)
Heavy ≥8 or ≥15 drinks/w	430	421 (97.9)	172	169 (98.3)	514	108 (21.0)	191	69 (36.1)
Smoking* **								
Never	206	195 (94.7)	483	469 (97.1)	393	61 (15.5)	891	176 (19.8)
Ex-smoker	69	68 (98.6)	20	18 (90.0)	273	29 (10.6)	71	10 (14.1)
Current-light 1-14g/d	308	293 (95.1)	82	78 (95.1)	348	53 (15.2)	83	25 (30.1)
Current-heavy ≥15g/d	92	89 (96.7)	15	15 (100)	103	13 (12.6)	6	2 (33.3)
Missing							2	0 (0.0)
Parity** **								
0-2			372	359 (96.5)			866	216 (48.00)
3 or more			153	148 (96.7)			1,436	193 (42.89)
Unknown			75	73 (97.3)			176	41 (9.11)
Contraceptive use** **								
Never oral (oc) or injectable (ic)			152	145 (95.4)			1,318	179 (39.78)
Ever oc never ic			49	46 (93.9)			271	35 (7.78)
Ever ic never oc			309	304 (98.4)			543	178 (39.56)
Ever ic and ever oc			88	83 (94.3)			332	56 (12.44)
#Ever ic and/ or ever oc			446	433 (97.1)			1,146	269 (59.78)
Missing			2	2 (100)			14	2 (0.44)
ART-period								
Pre-ART 1995-2004	162	144 (88.9)	146	138 (94.5)	1500	99 (6.6)	2,274	220 (9.7)
Early ART 2005-2009	229	227 (99.1)	229	226 (98.7)	895	121 (13.5)	1,848	334 (18.1)
Late ART 2010-2016	284	274 (96.5)	225	216 (96.0)	1002	159 (15.9)	2,790	684 (24.5)

Abbreviations: IQR, Interquartile range; oc, oral contraceptive; ic, injectable contraceptive.

#Ever ic and /or ever oc: a sum of category (2 to 4), ever oc never ic, ever ic never oc and ever ic and ever oc.

Total number of controls for the lifestyle exposures are different: for the demographic variables, infection unrelated cancer controls were used. For alcohol consumption, the controls comprised participants with cancers that were unrelated to infectious agents and alcohol. For smoking, the controls were not related to infectious agents and smoking. Contraceptive use controls were cancers unrelated to infectious agents and hormonal contraceptives. *Infection unrelated controls = 10,309 **Alcohol unrelated controls = 3,090 ***Smoking unrelated controls = 2,170 **** Contraceptive unrelated controls = 6,870

Table S4-4: Kaposi sarcoma cumulative incidence rate per 100 000 for black male and female South Africans: data source National Cancer Registry annual reports.

Year	Males	Females	Ratio
2001	392.15	172.35	1:2.28
2006	606.995	382.09	1:1.59
2016	416.4	215.9	1:1.93
2017	69.68	34.97	1:1.99

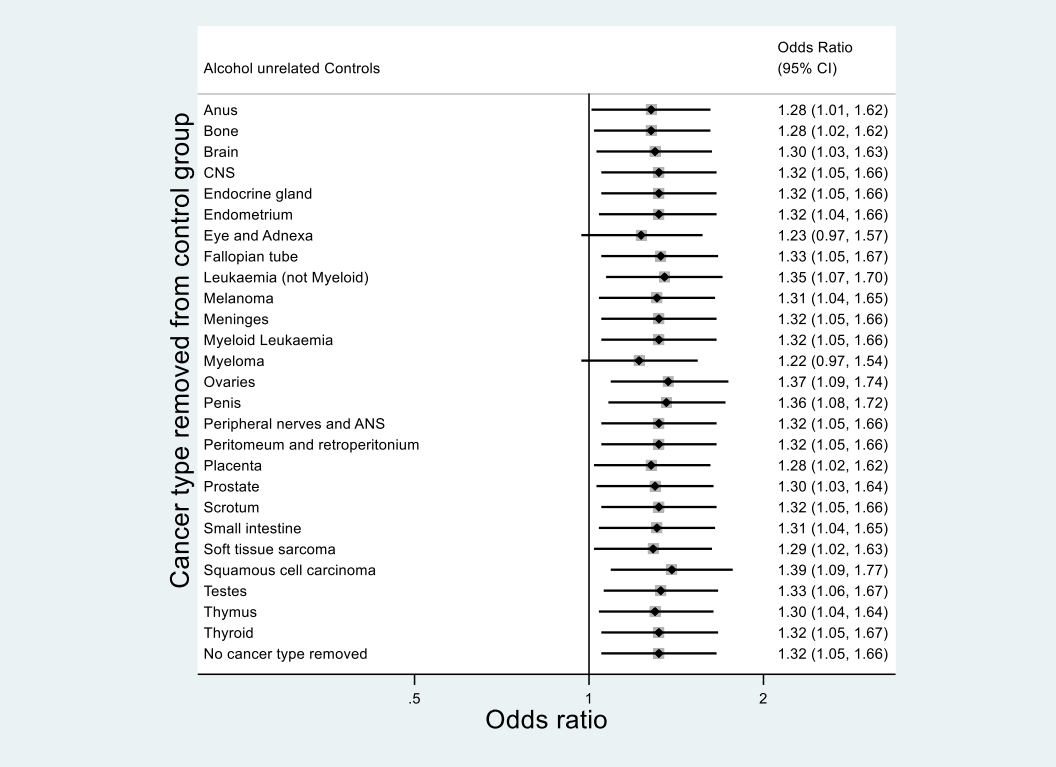

Figure S4-1: Adjusted OR (95% CI) for KS in relation to ever vs never alcohol consumption, demonstrating the effect of removal of single specific cancer types from the alcohol unrelated control group**.** Adjusted for age group, year of interview, education, alcohol consumption, number of sexual partners, urban/rural residence, ART period and HIV status. Squares represent ORs, and horizontal lines indicate 95% CI.

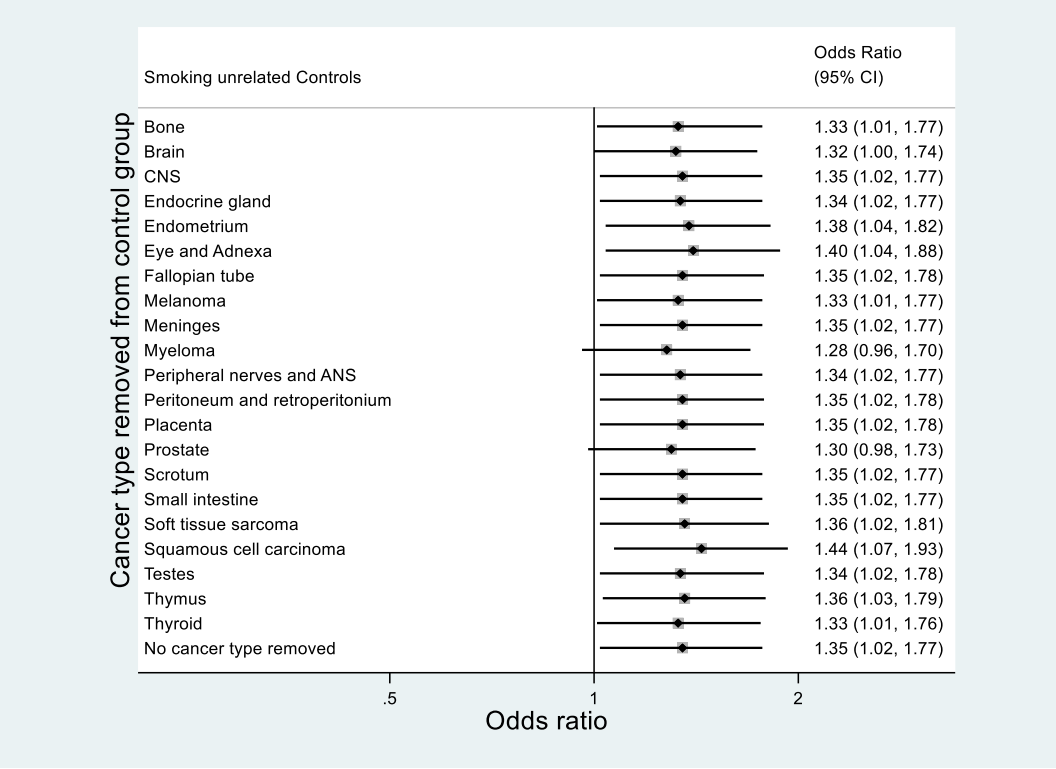

Figure S4-2: Adjusted OR (95% CI) for KS in relation to current vs never smoking, demonstrating the effect of removal of single specific cancer types from the smoking unrelated control group. Adjusted for age group, year of interview, education, smoking, number of sexual partners, urban/rural residence, ART period, and HIV status. Squares represent ORs, and horizontal lines indicate 95% CI.

Table S4-5: Adjusted OR to assess the effect of alcohol consumption and sex using alcohol unrelated cancer controls

Variable	**OR 95% CI**
Alcohol	
Never	1.00
Light drinker	1.33 (0.86 – 2.04)
Heavy drinker	**1.31 (1.03 – 1.67)**
Alcohol #sex*	
Never	1.00
Light drinker	1.23 (0.65 – 2.31)
Heavy drinker	1.37 (0.98 – 1.90)

*P-value 0.8252 (not significant)
Adjusted for sex, age group, year of interview, HIV status, education, number of sexual partners, place of residence and ART period.

Table S4-6: Adjusted OR to assess the effect of smoking and sex using smoking unrelated cancer controls

Variable	**OR 95% CI**
Smoking	
Never	1.00
Ex-smoker	1.00 (0.65 – 1.54)
Light-current	**1.41 (1.03 – 1.92)**
Heavy-current	**1.82 (1.06 – 3.10)**
Smoking#sex*	
Never	1.00
Ex-smoker	0.89 (0.53 – 1.48)
Light-current	1.45 (0.98 – 2.14)
Heavy-current	1.78 (0.98 – 3.20)

*P-value 0.6665 (not significant)
Adjusted for sex, age group, year of interview, HIV status, education, number of sexual partners, place of residence and ART period.

Chapter 5: Kaposi sarcoma-associated herpesvirus, HIV-1 and Kaposi sarcoma risk in black South Africans diagnosed with cancer during antiretroviral treatment rollout

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In this chapter we assessed the background seroprevalence of KSHV antibodies using infection unrelated controls. We measured the association between seropositivity to KSHV, HIV and the development of KS among black South Africans before and after ART rollout. The seroprevalence of KSHV, its relationship with HIV and the effect of ART is still unclear in the black population. Data was from the JCS, we used questionnaire data and serum collected from 1,237 Kaposi sarcoma cases and 6,649 infection unrelated controls (infection unrelated cancers). Seropositivity to KSHV antibodies was measured using a multiplex serological assay based on recombinant glutathione S-transferase (GST) capture immunoassay in combination with fluorescent beads on a Luminex platform.

5.1 Introduction

KS is caused by KSHV and is consistently associated with HIV (4). In 2020, GLOBOCAN reported 34,000 new cases and 15,000 deaths from KS, the majority arising in sub-Saharan Africa (2).

Molecular methods have been used to detect the presence of KSHV DNA in cells, tissues, and body fluids (160). Serological antibody detection methods against latent and lytic KSHV proteins include IFA, Western blots, and ELISA. KSHV in sub-Saharan African adults varies from 16% to 75%, which may be explained by different assays used (20). Earlier assays (161) focused on positivity to either latent or lytic antibodies (LANA encoded by ORF73 or the lytically expressed K8.1 encoded by ORF65) making accurate comparisons difficult, but recent assays can detect both (162). In South African adults the prevalence KSHV infection in adults ranges from 16 to 75% using IFA or ORF65 ELISA to detect latent antibodies (19–21). In other parts of the world, KSHV seroprevalence ranges between two and five percent (163). More recently a rapid multiplex immunoassay for detecting KSHV antibodies reported sensitivity of 95.1% and specificity of 91.4% in detecting LANA and K8.1 (162).

In black South Africans reported in the pathology-based National Cancer Registry the KS male to female ratio was 7:1 in 1988 (33) declining to 1.6:1 in 2019 (164). In Sub Saharan Africa males have a 23% increased odds of seropositivity to KSHV but females have about two-fold higher HIV seroprevalences (102,165) suggesting non-viral risk factors may be at play (32). Relative risks or odds ratios between KSHV and KS development range from over 10- to 100-fold, mainly in the context of HIV-1 infection (4). In Uganda, antibody titers to K8.1 and especially LANA increase significantly prior to a KS diagnosis (166).

Non-viral risk factors for KSHV infection prior to HIV-1 are based on small inconclusive studies, the most compelling being extended contact with water, number of sexual partners, and a history

of STDs (29,47,96,109,110,154). Using data from this study we showed an association between KS and alcohol and smoking (167).

The aims of the JCS described elsewhere (144) were to measure the relative importance of known and emerging risk factors for cancer in a black urban African population. Recruitment of people diagnosed with cancer began in 1995 and ended in 2016. In 1999, based on 51 cases of KS and 3,293 controls, using a first generation labor-intensive LANA IFA to detect KSHV, data from JCS showed an OR of 1,683 for KS in relation to HIV-1 positivity and high KSHV titers (19). Other studies on KSHV and HIV were based on small numbers of KS cases and reported that the risk of KS increased with increasing titer of antibodies against KSHV, independently of HIV infection (59,166). With 1,237 cases of KS recruited and 6,649 controls recruited over a 21-year period, and using high-throughput multiplex serology, we are now able to provide more reliable estimates on the relationship between KS and KSHV, HIV-1 and the association between KSHV and select demographic and lifestyle co-factors. Recruitment coincided with national ART rollout in 2004. We therefore also measured the prevalence of KSHV and HIV-1 in non-KS controls before and after ART rollout, and measured the association between HIV-1, KSHV and KS during this period.

5.2 Methods

5.2.1 Study setting and design

Between 1995 and 2016, the JCS recruited approximately 25,000 consenting black South Africans, mainly residents of Johannesburg/Soweto, newly diagnosed with any cancer aged between 18-74 years, referred to the medical oncology and radiation therapy departments of Charlotte Maxeke Johannesburg Academic Hospital (the largest in the region) from associated referral hospitals and

clinics. Participants were interviewed in English, Zulu or Sotho to collect sociodemographic and behavioral characteristics. Interviewers collected an optional venous blood sample in a serum separator tube (SST), aliquoted for routine HIV testing and future serological assays (in this case KSHV). Sera were stored at -25°C. Over 90% of tumors were microscopy/histopathology verified (27). Cancer types were coded to their primary site and morphology using the ICD-O3 and presented in ICD-10 format. Cases were participants with a new diagnosis of KS (C46) (N=1,237: 658 males and 579 females). Controls were participants diagnosed with cancer types not known to be infection-related (N=6,649: 2,288 males and 4,361 females). **Figure S5-1** outlines study numbers, exclusions, case and control selections for the study. **Table S5-1** shows the distribution of cancer types and KSHV seroprevalence in the controls.

5.2.2 Ethics

The University of Witwatersrand Human Research Ethics Committee (Medical) approved the primary study and the current study (Clearance certificate number: M191130).

5.2.3 Serology for HIV-1 and KSHV

HIV-1 antibody testing was performed locally by the National Health Laboratory Services using the Vironostika (HIV Uniform II plus O) micro-ELISA assay (27). Aliquots of sera were shipped on dry ice without any case/control identifiers to colleagues at the German Cancer Research Center (Deutsches Krebsforschungszentrum-DKFZ) in Heidelberg, who tested for antibodies against KSHV LANA and K8.1 using a multiplex serological assay (101) based on a glutathione S-transferase (GST) capture immunoassay in combination with fluorescent beads on a Luminex platform (101). The final net (bead and GST background subtracted) Median Fluorescence

Intensity (MFI) values were analysed at a dilution of 1:1000 and all values below +1 MFI were set to +1 MFI. KSHV seropositives were defined as positive for either LANA or K8.1. KSHV seronegatives were LANA and K8.1 negative (Table S5-2). Table S5-3 shows the distribution of the participants who had KSHV serology data versus those who did not.

5.2.4 Statistical Analysis

Aside from descriptive statistics, we examined the association between antibodies against KSHV by sex, HIV status, sociodemographic factors and ART periods. Prevalence odds ratios (OR) and 95% confidence intervals (CI) were calculated by unconditional unmatched logistic regression to identify risk factors associated with KSHV seropositivity among controls. We used standard case-control analysis methods (168) and similar regression models to measure adjusted case-control ORs of developing KS stratified by sex, for HIV and KSHV status. We stratified the KS ORs by HIV and KSHV status. Covariates were sex, age group (18-34, 35-44, 45-54, 55-64 and 65-74 years), HIV status, residence (urban/rural), education (none, primary (up to five years), secondary or higher) and number of sexual partners (none or one, two to five, six or more, missing). ART periods were classified as pre-ART (1995–2004), early ART (2005–2009) and late ART (2010–2016). National ART coverage in adults rose to 14.8% in 2009 (early ART period) and 48.6% in 2015 (late ART) (169). Statistical analyses were performed using STATA software V15.0 (Stata Corp, College Station, Tx). We used the Cochran Q-test (*admetan* function) to test for heterogeneity in ORs between sexes.

5.3 Results

5.3.1 KSHV Prevalence and risk factors in controls

Of 7,886 samples tested for KSHV, 2,958 were seropositive for LANA and 2,479 for K8.1 see **Table S5-2**. **Table 5-1** and **Figure 5-1** show the age distribution of KSHV seropositivity in controls peaking at 47% in those aged 65-74 years. Median age of KSHV positivity was 59 years in males and 53 in females. Participants with KSHV serology tended to be younger, more urbanised and had higher levels of education (**Table S5-3**).

The majority, 658 of the 1,237 individuals diagnosed with KS were male (**Table 5-1**). Cases were younger with a median age of 38 (IQR 33-44) in males and 33 (IQR 29-40) in females. Most of the KS cases were HIV positive (95.9% in males and 97.1% in females), HIV prevalence among the controls was 12.9% in males and 20.5% among females. Most of the KS cases were KSHV seropositive (98.5% males and 96.7% females), KSHV seroprevalence among controls was 32.7% in males and 29.6% in females.

KSHV seropositivity in controls (**Table 5-2**) was significantly associated with being male [OR_{adj}=1.37 (95%CI=1.23–1.52)], HIV positivity [OR_{adj}=4.21 (95%CI=3.74–4.73)] increased age group (Ptrend=0.014) and inversely associated with secondary or higher levels of education [OR_{adj}=0.70 (95%CI=0.59–0.83), p<0.001)]. Compared to pre-ART, we observe lower KSHV seroprevalences in relation to the early and late ART rollout periods [respectively: OR_{adj}=0.73 and OR_{adj}=0.68, P_{trend}<0.001]. By contrast, HIV prevalence among controls increased over the three ART periods from 7.8% to 15.5% in males and from 12.0% to 26.0% among females (P_{trend}<0.001). The proportion of high school leavers increased over time (**Table S5-4** and **Table**

S5-5). We found no association between KSHV seropositivity and residence or number of sexual partners.

5.3.2 Factors associated with KS

Table 5-3 shows the association between KS and KSHV, HIV and selected risk factors including ART rollout period. KS is strongly associated with KSHV antibody positivity (OR_{adj}=132.21; 95%CI=86.30–202.52); males were similarly likely to develop KS as females (respectively OR_{adj}=188.60, versus OR_{adj}=115.52, M/F $P_{heterogeneity}$=0.297). The odds of developing KS among seropositive LANA and K8.1 cases vs. controls were lower than results using the combined assay, but similar to each other: LANA OR_{adj}=61.5 and K8.1 OR_{adj}=64.1, $P_{heterogeneity}$=0.851]. KS is also significantly associated with HIV positivity ([OR_{adj}=86.96;95%CI=60.36–125.49] with no differences in risk between the sexes (Female OR_{adj}=86.04, Male OR_{adj}=83.39, M/F $P_{heterogeneity}$=0.934). There was no association between KS and residence, education, or number of sexual partners. There was a reduction in KS risk in relation to ART period in both sexes (P_{trend}=0.005). Similar ART trends in KS risk were observed for males and females, however the reductions in sample size reduced statistical power (P_{trend} males=0.16, females=0.073).

5.3.3 Association between KSHV, HIV antibody status and KS

Using those who were HIV and KSHV seronegative as reference (**Figure 5-2**), the odds of KS among those who were HIV seronegative and KSHV seropositive was 25-fold higher [OR_{adj}=25.47 (CI=10.54–61.50)]. The risk of developing KS in those who were KSHV and HIV seropositive increased by about 100-fold from 25 to 2500 [OR_{adj}=2501.10;CI=1083.10–5775.51, notably

OR_{adj}=2,035.10 in males and OR_{adj}=4,926.36 in females]. Twenty-three cases with KS were KSHV seronegative, possibly due to host or laboratory factors.

Table 5-1: Socio-demographic characteristics of study participants (cases and controls) by KSHV status

Characteristics	**Cases (n=1,237)**				**Controls (n=6,649)**			
	Males		**Females**		**Males**		**Females**	
	Total (658)	**KSHV seropositive N (%) 648 (98.48)**	**Total (579)**	**KSHV seropositive N (%) 560 (96.72)**	**Total (2,288)**	**KSHV seropositive N (%) 747 (32.65)**	**Total (4,361)**	**KSHV seropositive N (%) 1,289 (29.56)**
	N	**N (%)**	**N**	**N (%)**	**N**	**N (%)**	**N**	**N (%)**
Median Age (IQR)	*38 (33 – 44)*	*38 (33 – 44)*	*33 (29 – 40)*	*33 (29 – 40)*	*56 (49 – 64)*	*59 (51– 66)*	*50 (41 – 57)*	*53 (45 – 60)*
HIV status								
Negative	27	22 (81.48)	17	16 (94.12)	1,992	662 (33.23)	3,469	1,045 (30.12)
Positive	631 (95.90)	626 (99.21)	562 (97.06)	544 (96.80)	296 (12.94)	85 (28.72)	892 (20.45)	244 (27.35)
Age group (years)								
18-24	11	11 (100)	51	48 (94.12)	76	13 (17.11)	76	16 (21.05)
25-34	213	210 (98.59)	275	266 (96.73)	105	17 (16.19)	392	67 (17.09)
35-44	282	278 (98.58)	163	158 (96.93)	232	61 (26.29)	1,023	230 (22.48)
45-54	117	114 (97.44)	67	65 (97.01)	582	161 (27.66)	1,411	393 (27.85)
55-64	27	27 (100)	21	21 (100)	787	254 (32.27)	1,016	374 (36.81)
65-74	8	8 (100)	2	2 (100)	506	241 (47.63)	443	209 (47.18)
Place of residence								
Urban	630	622 (98.73)	543	524 (96.50)	2,073	669 (32.27)	3,964	1,155 (29.14)
Rural	26	25 (96.15)	34	34 (100)	209	76 (36.36)	387	129 (33.33)
Missing	2	1 (50.00)	2	2 (100)	6	2 (33.33)	10	5 (50.00)
Education								
None	30	29 (96.67)	24	23 (95.83)	263	120 (45.63)	425	164 (38.59)
Primary	150	148 (98.67)	90	89 (98.89)	833	305 (36.61)	1,166	419 (35.93)
Secondary & Tertiary	478	471 (98.54)	465	448 (96.34)	1,187	321 (27.04)	2,764	705 (25.51)
Missing					5	1 (20.00)	6	1 (16.67)
Number of sexual partners								
0-1	16	15 (93.75)	24	23 (95.83)	100	27 (27.00)	403	130 (32.26)
2-5	205	202 (98.54)	321	306 (95.33)	771	275 (35.67)	2,476	727 (29.36)
6 or more	256	252 (98.44)	108	105 (97.22)	792	276 (34.85)	471	140 (29.72)
Unknown	181	179 (98.90)	126	126 (100)	625	169 (27.04)	1,011	292 (28.88)
ART period								
Pre-ART 1995 – 2004	157	152 (96.82)	138	129 (93.48)	653	260 (39.82)	1,180	416 (35.25)
Early ART 2005 – 2009	226	224 (99.12)	228	221 (96.93)	687	214 (31.15)	1,407	374 (26.58)
Late ART 2010 – 2016	275	272 (98.91)	213	210 (98.59)	948	273 (28.80)	1,774	499 (28.13)

Table 5-2: Univariate and multivariate logistic regression analysis of risk factors of KSHV infection in controls (for breakdown see **Table S5-2**).

Variables	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Sex		
Males	**1.50 (1.37 – 1.65)**	**1.37 (1.23 – 1.52)**
Females	1.00	1.00
P-heterogeneity		<0.001
HIV status		
Negative	1.00	1.00
Positive (all ages)	**3.66 (3.31 – 4.05)**	**4.21 (3.74 – 4.73)**
P-heterogeneity		0.004
Age group (years)		
18-24	1.00	1.00
25-34	**1.89 (1.40 – 2.55)**	1.35 (0.98 – 1.86)
35-44	1.07 (0.80 – 1.43)	0.92 (0.67 – 1.25)
45-54	0.73 (0.55 – 0.97)	0.82 (0.60 – 1.12)
55-64	0.82 (0.62 – 1.10)	1.07 (0.78 – 1.46)
65-74	1.32 (0.98 – 1.78)	**1.84 (1.33 – 2.55)**
P-trend		0.014
Place of residence		
Urban	1.00	1.00
Rural	0.96 (0.82 – 1.13)	0.98 (0.82 – 1.17)
P-heterogeneity		0.611
Education		
None	1.00	1.00
Primary	0.91 (0.77 – 1.07)	0.86 (0.72 – 1.03)
Secondary & Tertiary	**0.80 (0.68 – 0.93)**	**0.70 (0.59 – 0.83)**
P-trend		<0.001
Number of sexual partners		
0-1	1.00	1.00
2-5	1.19 (0.99 – 1.44)	1.04 (0.85 – 1.26)
6 or more	**1.62 (1.32 – 1.97)**	1.16 (0.93 – 1.45)
Unknown	1.16 (0.95 – 1.42)	1.00 (0.77 – 1.25)
P-trend		0.762
ART period		
Pre-ART 1995 – 2004	1.00	1.00
Early ART 2005 – 2009	**0.78 (0.71 – 0.86)**	**0.73 (0.64 – 0.82)**
Late ART 2010 – 2016	**0.74 (0.68 – 0.81)**	**0.68 (0.60– 0.78)**
P-trend		<0.001

Adjusted for sex, HIV status, age group, ART-period, place of residence, level of education and number of sexual partners.

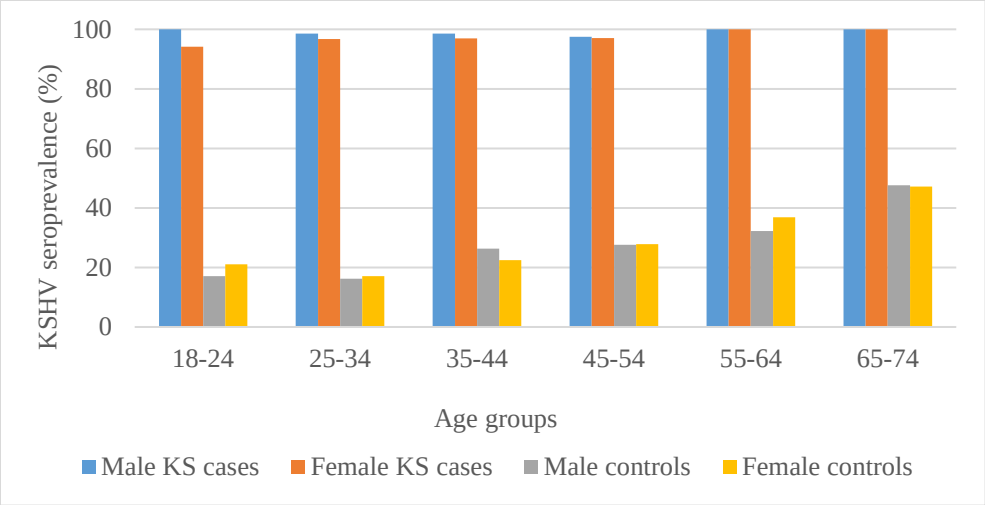

Figure 5-1: Age-associated seroprevalence of KSHV antibodies in KS cases and controls.

Table 5-3: Case-control risks of developing KS stratified by sex.

Variables	**Adjusted OR (95%CI)**		
	Both sexes	**Males**	**Females**
KSHV status			
Negative	1.00	1.00	1.00
Positive	**132.21 (86.30 – 202.52)**	**188.60 (88.36 – 402.57)**	**115.52 (68.56 – 194.66)**
P-heterogeneity		0.297	
Positive (LANA only)	**61.50 (44.86 – 84.35)**		
Positive (K8.1 only)	**64.12 (47.47 – 86.63)**		
P-heterogeneity	0.851		
HIV status			
Negative	1.00	1.00	1.00
Positive	**86.96 (60.36 – 125.29)**	**83.39 (50.21 – 138.51)**	**86.04 (50.26 – 147.30)**
M/F Cochran Q P-value		0.934	
Place of Residence			
Urban	1.00	1.00	1.00
Rural	1.05 (0.62 – 1.80)	1.17 (0.50 – 2.78)	1.09 (0.54 – 2.19)
Education			
None	1.00	1.00	1.00
Primary	0.76 (0.45 – 1.30)	1.17 (0.52 – 2.61)	0.59 (0.29 – 1.19)
Secondary & Tertiary	0.97 (0.58 – 1.63)	1.63 (0.73 – 3.64)	0.74 (0.38 – 1.45)
No. of sexual partners			
0-1	1.00	1.00	1.00
2-5	1.18 (0.62 – 2.26)	0.95 (0.26 – 3.51)	1.21 (0.56 – 2.61)
6 or more	1.23 (0.63 – 2.43)	1.12 (0.30 – 4.14)	1.27 (0.55 – 2.93)
Unknown	1.22 (0.60 – 2.46)	1.57 (0.40 – 6.15)	1.02 (0.44 – 2.37)
ART-period			
Pre-ART 1995-2004	1.00	1.00	1.00
Early ART 2005-2009	0.84 (0.60 – 1.19)	0.69 (0.39 – 1.22)	0.95 (0.61 – 1.47)
Late ART 2010-2016	**0.64 (0.45 – 0.93)**	0.65 (0.35 – 1.19)	0.65 (0.40 – 1.04)
P-trend	0.005	0.160	0.073

Adjusted for HIV status, KSHV status, age group, place of residence, education, number of sexual partners and ART period.

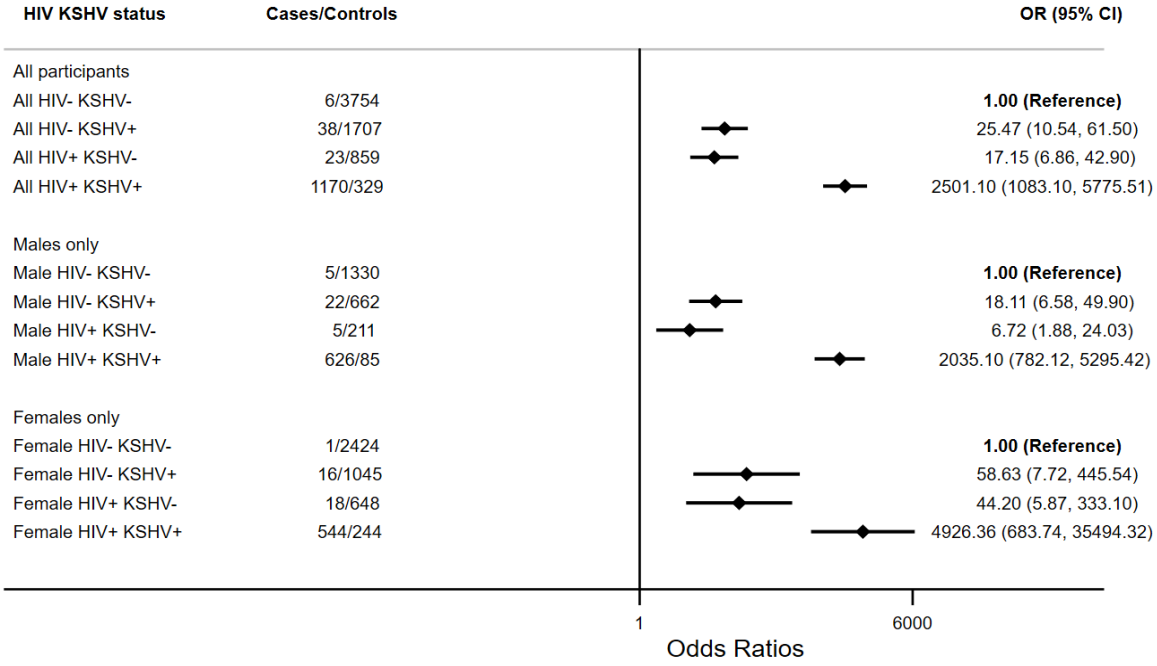

Figure 5-2: The relationship between HIV and KSHV seropositivity and KS risk. Odds Ratios adjusted for age, education, and number of sexual partners.

5.4 Discussion

In the previous study using JCS data, the immunofluorescence assay used had a sensitivity of 60-80% in detecting KSHV antibodies (19). In this case the serological assay detected weak antibody responses against the KSHV LANA and K8.1. Assuming all KS cases to be KSHV positive, this assay shows a sensitivity of 97.7%. Making comparisons of seroprevalence between different studies can be problematic because of the different serologic assays that evolved over time (35). However, our findings on the seroprevalence of antibodies against KSHV and age, maleness, lower education level and associations with HIV-1 are consistent with other studies from Africa (19,62,170).

The higher KSHV seroprevalence with in older ages (31% overall, 20% in 18–34-year-olds, 47% in those aged 65-74) suggests that KSHV may have been prevalent in this population for some time (19) or due to improvements in seroprevalence in relation to age (generation/cohort effect) (17,54). These findings are consistent with the findings from previous studies from South Africa reporting high seropositivities of KSHV (19–21). Using LANA and K8.1 assays separately yielded lower but similar risks, but we could not replicate longitudinal data from Uganda showing an increase in Lytic antibody levels prior to KS development (166).

Given the causal relationship between KSHV, HIV and KS the seroprevalence of both viruses among participants with KS was over 95%. There was a decline in KS incidence after ART rollout which can be attributed to reduction in immune suppression among KS cases as seen in South Africa (90) and elsewhere in Africa (171). This contrasts with HIV seroprevalence, which

increased from 10% in the pre-ART period to 22% in the late-ART period, in keeping with improved post-HIV survival due to ART.

The study confirms previous findings (19) of the strong association between HIV and KS (about 80-fold), and KSHV and KS (ORs of 132-fold) with no statistically significant difference in risk between the sexes. When compared to HIV and KSHV negatives, HIV seropositivity had about a 100-fold effect on KS risk, increasing risks from 25-fold in KSHV+ and HIV- patients to 2500-fold in doubly infected (HIV+, KSHV+) patients. The risk for developing KS was higher in doubly infected females (5000-fold) compared to males (2000-fold). While KSHV is (a little) more common in male controls, the ORs observed for two major viral factors responsible for KS development cannot explain the observed 1.5- to 2-fold male predominance in KS incidence.

Our observed decline in KSHV seropositivity from about 35% pre-ART rollout to 28%. We observed a decrease seropositivity post introduction of ART but one study in the United States of America and Puerto Rico reported an increase in KSHV transmission over a ten year period (172). However, as the bulk of KSHV transmission in Africa appears to be from person to person, improved hygiene conditions could also be responsible for the decline in KSHV as illustrated by a modest increase in number of high school leavers observed over time. We did not collect income data but the proportion using electricity for cooking increased from 70% in the first half of the study to 84% in the second half (144).

5.5 Limitations

Samples were tested for antibodies at the time of cancer diagnosis and the tumor may influence antibody titers. However, we found little evidence of this. The prevalence of KSHV is similar across all the cancer types constituting the controls (**Table S5-2**) and resemble those from independent studies. Study results are based on participants recruited from the catchment area of the largest tertiary hospital in Johannesburg/Soweto. Therefore, our findings might not be generalizable to other populations. Recall of past exposures such as numbers of sexual partners could be subject to recall or desirability bias. However, given all participants are patients with cancer we expect similar recall across cases and controls. Participants with KSHV serology were younger and more educated, however in all analyses we adjusted for both factors.

5.6 Strengths of the study

This study was conducted on a large sample size compared to previous studies (19,20,62). The laboratory tests were performed without knowledge of the case/control status of the participants. Case-control referral biases are also minimized because both groups come from the same catchment areas and are referred through the same processes and departments. Over 90% of the tumors were histologically verified (27). Controls for this analysis were carefully selected, comprising cancer types not known in the literature to be associated with infections (144).

5.7 Conclusion

Our findings highlight the high seroprevalence of antibodies against KSHV and the striking association between KSHV, HIV and KS found in African settings. ART rollout coincides with a decline in KSHV seroprevalence, in keeping with a decline in KS incidence after ART rollout (90).

By contrast we did not observe any declines in HIV prevalence over the same period. Our observed higher KS risks in KSHV and HIV seropositive females cannot explain the higher population male to female KS incidence ratios.

Treating underlying immune deficiency is the current way by which KS can be managed, however better targeted therapies are called for, and better still a vaccine. Herpesviruses (of which KSHV is one of nine) are a serious complication in patients with HIV so ideally, aside from adherence to ARVs, a safe and effective herpes-virucidal agent could be explored. Having said that, identifying immune responses in the general population and co-factors predictive of KS disease are important areas to mitigate disease onset. Among HIV positive patients, even though the risks of developing KS in those who test KSHV positive are large, this KSHV test yields a sensitivity of 99%, but a specificity of 53%, (from **Figure 5-2**) so a more specific KSHV test needs to be developed for it to be clinically useful in detecting KS in this high KSHV prevalence setting.

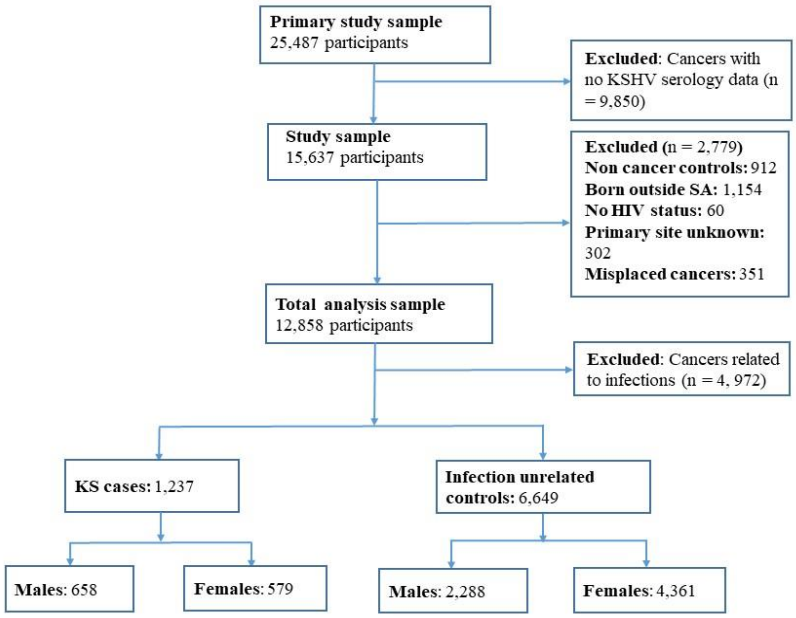

Figure S5-1: Selection of study participants (cases and infection unrelated controls).

Table S5-1: Seroprevalence of KSHV among unrelated cancer controls (both sexes, males, and females).

Infection unrelated cancer controls (ICD-O-3-code)	**Total N**	**KSHV seropositive All participants**	**All males N**	**KSHV seropositive Males**	**All females N**	**KSHV seropositive Females**
Bone (C40-41)	100	26 (26.00)	55	17 (30.91)	45	9 (20.00)
Breast (C50)	2,359	588 (24.93)	-	-	2,359	588 (24.93)
Colon (C18-20)	654	196 (29.97)	296	91 (30.74)	358	105 (29.33)
Endometrium (C54-55)	388	159 (40.98)	-	-	388	159 (40.98)
Kidney (C64)	60	20 (33.33)	36	11 (30.56)	24	9 (37.50)
Larynx (C32)	334	133 (39.82)	296	109 (36.82)	38	24 (63.16)
Lung (C33-34)	895	312 (34.86)	684	226 (33.04)	211	86 (40.76)
Melanoma (C43)	56	15 (26.79)	12	1 (8.33)	44	14 (31.82)
Myeloid leukemia (ICD-10 C92)	251	75 (29.88)	122	37 (30.33)	129	38 (29.46)
Myeloma (C90)	187	52 (27.81)	73	18 (24.66)	114	34 (29.82)
Esophagus (C15)	387	126 (32.56)	243	77 (31.69)	144	49 (34.03)
Ovaries (C56)	310	118 (38.06)	-	-	310	118 (38.06)
Pancreas (C25)	174	57 (32.76)	104	35 (33.65)	70	22 (31.43)
Prostate (C61)	296	110 (37.16)	296	110 (37.16)	-	-

Infection unrelated cancer controls (ICD-O-3-code)	Total N	KSHV seropositive All participants	All males N	KSHV seropositive Males	All females N	KSHV seropositive Females
Other minor cancers* (brain (C71), CNS (C72), endocrine gland (C75), fallopian tube (C57.0), meninges (C70), peritoneum and retroperitoneum (C48), placenta (C58.9), scrotum (C63.2), small intestines (C17), testes (C62), thyroid (C73))	198	49 (24.75)	71	15 (21.13)	127	34 (26.77)
Total	6,649	2,036 (30.66)	2,288	747 (32.65)	4,361	1,289 (29.56)

*Minor cancers sum of cancer types with less than 50 participants.

Table S5-2: LANA and K8.1 seropositivity.

LANA	K8.1		Total
	Seronegative	Seropositive	
Seronegative	4,642	286	4,928
Seropositive	765	2,193	2,958
Total	5,407	2,479	7,886

Table S5-3: The distribution of the participants who did not have any KSHV serology versus those with serology.

Variables	Participants without serology (N=9,850) N (%)	Participants with serology (N= 7,886). N (%)
Mean age	56 (48-63)	50 (40-58)
Place of residence		
Urban	8,300 (84.26)	7,210 (91.43)
Rural	1,494 (15.17)	656 (8.32)
Missing	56 (0.57)	20 (0.25)
P-value	<0.001	
Education		
None	1,674 (16.99)	742 (9.41)
Primary	3,676 (37.32)	2,239 (28.39)
Secondary	4,454 (45.22)	4,894 (62.06)
Missing	46 (0.47)	11 (0.14)
P-value	<0.001	

Table S5-4: HIV prevalence among cases and infection unrelated control over the years (ART periods) by sex.

ART period	Male Cases	HIV pos N (%)	Female cases	HIV Pos N (%)	Male controls	HIV Pos N (%)	Female controls	HIV Pos N (%)
Pre-ART 1995 – 2004	157	141 (89.81)	138	133 (96.38)	653	50 (7.66)	1,180	141 (11.95)
Early ART 2005 – 2009	226	225 (99.56)	228	225 (98.68)	687	99 (14.41)	1,407	289 (20.54)
Late ART 2010 – 2016	275	265 (96.36)	213	204 (95.77)	948	147 (15.51)	1,774	462 (26.04)
P-value		<0.001		0.168		<0.001		<0.001

Table S5-5: HIV and KSHV seroprevalence among controls over the years (ART periods).

ART period	All controls	HIV pos N (%)	KSHV pos N (%)	Level of education (Secondary or higher) N (%)
Pre-ART 1995 – 2004	1,833	191 (10.42)	676 (36.88)	884 (48.46)
Early ART 2005 – 2009	2,094	388 (18.53)	588 (28.08)	1,230 (58.80)
Late ART 2010 – 2016	2,722	609 (22.37)	772 (28.36)	1,837 (67.49)
Total	6,649	1,188 (17.87)	2,036 (30.62)	3,951 (59.52)
P-value		<0.001	<0.001	<0.001

PART 3: GENERAL DISCUSSION

Chapter 6: Discussion

This chapter is a consolidation of findings with regards to key objectives of this thesis. It also comprises of reflections on the key findings, contextual relevance, the strengths and limitations, future research, conclusion and recommendations.

6.1 Summary of study findings

The aim of this study was to describe the epidemiology of Kaposi sarcoma in the adult (18-74 years) black South African population. The specific objectives were to evaluate the KS incidence rates and assess the effect of ART on KS in sub-Saharan Africa; to assess whether the lower incidence of KS in females than males is related to any of the lifestyle factors; to assess the seroprevalence of KSHV, among infection unrelated controls and to examine the relationship between KSHV and HIV and KS. The consolidated findings of the study are outlined in **Table 6-1**.

Table 6-1: Consolidated study findings.

Chapter	Objective	Key findings
3	1. To evaluate the KS incidence rates and assess the effect of ART on KS in sub-Saharan Africa.	-There is a significant under-reporting of cancer incidence in sub-Saharan Africa as there are few population-based cancer registries in the region. -ART rollout coincides with a decline in KS incidence across several regions in sub-Saharan Africa.
4	2. To assess whether the lower incidence of KS in females than males is related to any of the lifestyle factors (e.g. number of sexual partners (male and female), hormonal contraceptive use and parity (females only), smoking and alcohol consumption).	-Smoking and alcohol consumption may provide a possible explanation for the KS sex differences, given both exposures are more common in men, but confounding and bias cannot be fully ruled out. -The role smoking and alcohol play in relation to viral loads of HIV/KSHV, differences in immunological responses or other genetic differences between males and females warrant further studies.
5	3. To evaluate the relationship between KSHV, HIV and KS and to assess the seroprevalence of KSHV among infection unrelated study controls, during antiretroviral treatment rollout	-The seroprevalence of antibodies against KSHV in a black South African population is high. -The current study reconfirms the striking association between KSHV, HIV and KS found in African settings. -ART is associated with a decline in KSHV seroprevalence, in keeping with a decline in KS incidence after ART rollout

6.2 Emerging research themes from the findings

Based on the consolidated findings of this thesis and the relevant literature, the emerging themes are: (1) the introduction of ART resulted in a decline in KS incidence in sub-Saharan Africa, (2)

smoking and alcohol consumption may provide a possible explanation for the KS sex differences, and (3) KSHV seroprevalence is high and HIV increases the risk of developing KS among those who were KSHV seropositive and ART is associated with a decline in KSHV seroprevalence.

6.2.1 ART resulted in a decline in KS incidence in sub-Saharan Africa

There is a significant under-reporting of cancer incidence in sub-Saharan Africa. There are few population-based cancer registries in the region. From the limited available data on population-based KS age standardized incidence rate there is a decline in KS incidence post ART rollout. ART use is effective in preventing KS and used in KS management in sub-Saharan Africa. Other factor that could be contributing to the decline include changes in the HIV prevalence over time. There are inconsistencies in the ART coverage across the region. Scaling up ART enrolment for PLWH and accurate, timely reporting remains a priority. The decline in KS due to ART highlights the importance of controlling oncogenic infections and immune restoration as cancer prevention and has implications for cancer control in general.

6.2.2 Lifestyle risk factors associated with KS sex differences ratios

Smoking and alcohol consumption may provide a possible explanation for the increased KS risk in males compared to females, given both exposures are more common in men, but confounding and bias cannot be fully ruled out. There is limited data on male KS dominance. Some viral diseases are more common in males than females and there are different health seeking behaviors among males and female. It is also possible that there are other risk factors and interactions that could explain the sex differences. The role smoking and alcohol play in relation to viral loads of HIV/KSHV, differences in immunological responses or other genetic differences between males and females warrant further studies.

6.2.3 KSHV, HIV and KS risk

The KSHV seroprevalence is high among adult black South Africans. We found KSHV seropositivity declined significantly in relation to the latest ART rollout period. This could suggest some level of KSHV viral clearance at a population level. There is an association between KSHV, HIV and KS. ART is associated with a decline in KSHV seroprevalence and a decline in KS incidence after ART rollout. ART rollout period also coincides with modest improvements in educational standard, which in turn may have an additional effect on KSHV prevalence and transmission. The current ART guidelines allow PLWH to go onto ART upon diagnosis. ART is the current way by which KS is managed. Even without a vaccine it may be possible to reduce the spread of KSHV through public health measures. It is important to educate PLWH to be able to access health care earlier and be on the lookout for early presentation of KS development for early detection. Educating the public on routes of transmission, could reduce new infection.

6.3 Limitations

The availability of population-based KS, HIV and ART data and the use of different data sources to reflect the population KS trends in sub-Saharan Africa made it difficult to ascertain the overall effect of ART on KS. Our studies on the lifestyle risk factors associated with KS incidence sex differences and KSHV, HIV and KS risk must be considered considering possible limitations inherent in case-control studies and using participants with a range of other cancers as controls. We carefully selected controls unrelated to exposures of interest to allow for valid comparisons. Recall of exposures by participants could be subject to recall or desirability bias. However, given all cases and controls are patients with cancer we expect recall to be similar across cases and controls, and in this case may attenuate any relationships. Referral bias cannot be ruled out as a

number of individuals who were treated successfully at e.g. dermatology clinics were not referred to the oncology departments, where the study participants were recruited. There was no ART adherence information collected so we could not account for how long the participants have been on ART. Our findings were based on the adult black South African population from Johannesburg and the findings might not be generalizable to other South African provinces and other population groups. Despite these limitations, our findings remain an important source to understand sex disparities in KS among the adult black South African population.

6.4 Strengths

The study used data from a large epidemiological study. Both cases and controls are people with incident cancers, thus interviewer and recall bias were minimized by interviewing cases and controls with diseases of similar severity. Referral biases are also minimized because both cases and controls are likely to be come from the same catchment areas and referred through similar clinic pathways. Over 95% of the cancers were histologically verified. Controls for this analysis were carefully selected, comprising cancer types not known to be associated with the exposures of interest (infectious agents, alcohol consumption smoking and hormonal contraceptive use). The control selection was robust, and for each analysis we performed extensive sensitivity analyses of the controls used. We used a recent high throughput multiplex serology method to detect the antibodies against KSHV which was more sensitive than previous methods.

6.5 Future research

Based on the findings from this thesis, future research has to:

1. Confirm the estimates of KSHV seroprevalence in adults in Johannesburg to reinforce the rationale to prioritise KSHV screening for early detection of KS.

2. Evaluate the feasibility of KSHV screening in clinical settings.

3. Investigate the differences in immunological responses or other genetic differences between males and females to further account for the KS sex difference ratios.

4. Evaluate whether smoking and alcohol act independently or whether they modify the role of HIV and KSHV on KS risk.

6.6 Summary conclusion

In sub-Saharan Africa, we showed, using limited evidence, a decline in KS incidence after the rollout of ART in most countries. Nevertheless, KS continues to be a major public health problem in terms of morbidity and mortality. HIV positivity increased the risk of epidemic KS and the risk was higher among females compared to males, suggesting viral factors alone cannot explain the uneven male KS ratios. We found increased risks of KS (in both sexes combined) in relation to smoking and alcohol consumption. Limitations aside (discussed above) these two factors could be at least partly responsible for the uneven KS sex ratios, given drinking and smoking is three to five times more common in males. Confirmatory studies are therefore needed. ART is associated with a decline in KSHV seroprevalence, and a decline in KS incidence after ART rollout. In high risk KSHV settings the specificity of KSHV serology needs to improve before it is used to identify individuals at high risk of KS improve management outcomes.

6.7 Recommendations

Sub-Saharan Africa is characterized by underreporting of cancer incidence data. We show here that tracking the emerging trends on ART by utilizing the incidence data is an essential tool that can assist with developing policies that include cancer-control, planning, early detection, and

prevention efforts. There is a need for the development of population-based cancer registries in sub-Saharan Africa.

Our study highlights the need to raise awareness about KS and HIV among clinicians and PLWH. This can be done through Information Education Communication (IEC) materials. Information on symptoms and signs of KS and risk factors for KS presented on posters, flyers, leaflets, brochures etc, could increase awareness among clinicians and PLWH. Encouraging clinicians to incorporating skin checks for lesions on the skin post HIV diagnosis during routine check-ups may increase early detection, since there is no screening tool for KS. PLWH need to know what to look out for as KS lesions present differently for different races and are painless, this may otherwise enable the diagnose of KS in a timely manner and better management outcomes.

The reasons for the observed sex differences observed in this study are likely manifold and could partially be attributed to alcohol consumption and smoking. The underlying causes for the sex differences remains poorly understood and should form basis for future investigations. We show here that smoking and alcohol consumption increase the risk of developing KS. IEC on risk factors could promote lifestyle modifications that could result in reduced transmission of KSHV and reduced KS incidence.

Our study show that ART is associated with decline in KSHV seroprevalence and a decline in KS incidence. This calls for continued improvement of ART coverage in sub-Saharan Africa, encouraging the public to test for HIV, enrol for treatment and adhere to treatment. More studies are therefore needed to ascertain the effect of ART on KSHV seroprevalence, and KS incidence

over time. Lastly, findings of the epidemiological trends of KS will permit clinicians and policy makers to prepare and adapt accordingly in terms of resource allocation, public health perspectives and formulation of management protocols and guidelines for patients with KS.

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8.0 Appendices

8.1 Appendix 1: Primary study questionnaire

MRC/NHLS/Wits CANCER EPIDEMIOLOGY RESEARCH GROUP (CERG)
JOHANNESBURG STUDY QUESTIONNAIRE FOR PATIENTS

Date of Birth OR Age Study No:
Day Month Year 1 9 Years Sex *(please tick)* 1 M F 2

Diagnosis(Clinical) Path No ICD

Histology result, if available Hospital No. M

Initials of Interviewer ..... Date of Interview Day Month Year 2 0

BLOODS:
RED TOP yes no
PURPLE TOP yes no

Where were you born? *(please tick)*
1 Soweto 2 Jhb 3 Gauteng 4 N.Prov. 5 Mpum 6 NW 7 FS 8 ECape
9 W.Cape 10 N.Cape 11 Kwazulu Natal 12. Other ..... 1 Urban 2 Rural

Where do you normally live? *(please tick)*
1 Soweto 2 Jhb 3 Gauteng 4 N.Prov. 5 Mpum 6 NW 7 FS 8 ECape
9 W.Cape 10 N.Cape 11 Kwazulu Natal 12. Other ..... 1 Urban 2 Rural

How long have you lived there? Years

What is the highest standard you passed at school? *(please tick)*

1	2	3	4	5	6	7	8	9	10	11	12	13	14
None	Sub A Gr1	Sub B Gr2	Std 1 Gr3	Std 2 Gr4	Std 3 Gr5	Std 4 Gr6	Std 5 Gr7	Std 6 Gr8	Std 7 Gr9	Std 8 Form 3 NTC1	Std 9 Form 4 NTC2	Std 10 Form 5 NTC3	Univ tech

What are the walls of your home made of? *(please tick)*
1. brick/cement 2. Tin 3. Plastic 4. Wood 5. Other (specify) ..... 6. mud/clay

Where do you normally cook food in your house? 1 Inside Outside 2

What type of fuel do you MOSTLY use in your home for cooking? *(please tick)*
1. Wood 2. Charcoal 3. Coal 4. Anthracite 5. Paraffin 6. Gas 7. Electricity 8. Other ..... 9. dung

What type of fuel do you MOSTLY use in your home for keeping warm? *(please tick)*
1. Wood 2. Charcoal 3. Coal 4. Anthracite 5. Paraffin 6. Gas 7. Electricity 8. Other ..... 9. dung 10. no fuel

Where did you normally cook food in your house *20 years ago*? 1 Inside Outside 2

What type of fuel did you MOSTLY use in your home for cooking *20 years ago*?
1. Wood 2. Charcoal 3. Coal 4. Anthracite 5. Paraffin 6. Gas 7. Electricity 8. Other ..... 9. dung

What type of fuel did you MOSTLY use in your home for keeping warm *20 years ago*?
1. Wood 2. Charcoal 3. Coal 4. Anthracite 5. Paraffin 6. Gas 7. Electricity 8. Other ..... 9. dung 10.no fuel

In any of the houses you lived in, did the smoke ever make your eyes water?
1 Yes, more than 5 years No 2

Have you ever smoked cigarettes or a pipe regularly? *(please tick)* 1. Yes(now) 2. In the past 3. Never

If yes: In the past five to ten years, how many would you usually smoke in a day?

	1. Cigarettes	2. Hand rolled cigarettes	3. Pipes	4. Dagga
(number of)				

How old were you when you first started smoking regularly? Years old

If you have stopped smoking, how old were you when you stopped? Years old

Have you ever used snuff? *(please tick)* 1. Yes(now) 2. In the past 3. Never

In the past five to ten years, how often would you use snuff each day? times per day

Before you became ill, did you ever have your blood pressure measured? *(Please tick)* 1. Yes 2. No 3. Yes only during pregnancy

If yes, were you told it was high? *(Please tick)* 1. Yes 2. No 3. Yes only during pregnancy

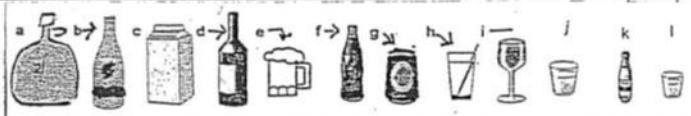
Have you ever had diabetes? *(Please tick)* 1. Yes 2. No

22 January 2010

Before you became ill, about how much wine, beer or spirits did you drink on average each week?
(Please indicate glass/bottle size when you select block for each type of drink - eg. 3 large glasses of maize beer per week would be: 3 e if none then 0

BEER			SPIRITS		WINE	CIDER; other	OTHER
sorghum (cartoon)	homemade (specify type)	commercial	Homemade	commercial		commerical alcoholic fruit drinks	SPECIFY

a: 2l (wine bottle)
b: bottle (1 litre)
c: 1l carton
d: 750ml (wine bottle)
e: 500ml (beer glass)
f: 375ml (half jack/pint)
g: 340ml (beer can/dumpy)
h: 250ml glass
i 200ml (wine) glass/nip
j : 100ml (small glass)
k 50ml (miniature airplane bottle)
l 25ml (tot)
mother (please specify)



Are you? *(please tick)*
1. ☐ Single (never been married) 2. ☐ Married(living as married) 3. ☐ Widowed 4. ☐ Separated

How many husbands/wives have you had?

FOR WOMEN ONLY: Have you ever been pregnant? *(please tick)* 1 Yes No 2

If yes, how many times have you been pregnant? ☐☐

If yes, how many times have you had a miscarriage? ☐☐

If yes, how many of your children were born alive? ☐☐

FOR MEN ONLY: How many children *(dead or alive)* do you have?

ALL: How many mothers / fathers do the children have?

ALL: How old is your oldest child now? *(include dead & alive)* ☐☐ Years old

ALL: How old is your youngest child now? *(include dead & alive)* ☐☐ Years old

ALL: How many boyfriends/girlfriends have you had?

FOR WOMEN ONLY
How old were you when your periods began? ☐☐ Years old

Age now (from previous page) _____

Age at first birth _____

Age at last birth _____

Have your periods ended? Yes ☐1 No ☐2 Not sure ☐3 If yes, how old were you when they ended? ☐☐ years old

If Yes, did your periods stop: ☑

1. naturally	2. due to surgery	3. due to medical treatment	4. due to IC use	5. other	6. unknown

	Oral	Injectable
Have you ever taken contraceptives?	1 Yes No 2	1 Yes No 2
If yes, how old were you when you started taking them?	☐☐	☐☐ Years old
How old were you when you stopped taking them?	☐☐	☐☐ Years old
If yes, for how long in total did you take them?	☐☐	☐☐ Years
Interruptions? ☑	1 Yes No 2	1 Yes No 2

Main reason for stopping, the last time you stopped using the method: ☐☐ ☐☐

1 Nausea 2 high BP 3 headaches 4 wt.Gain 5.changed menstrual pattern 6. menopause 7.sterilization 8.hysterectomy 9. no partner 10. to become pregnant 11 other 0.no specific reason

Have you ever had a 'pap' smear?☑

0. Never	1. Yes	2. current illness only	9. Can't remember		If yes, how many?	Age at first pap(yrs)	Age at last pap (yrs)

FOR ALL: Describe your usual or past **occupation** (eg. Driver, machine operator).....

Describe what your usual **workplace** does or did (eg. A bank, a chemical factory, a gold mine.....

What language do you speak most at home? ☐

What language does/did your father speak? ☐

What language does/did your mother speak? ☐

1. Zulu 2. Xhosa 3. Sotho 4.Pedi 5. Tswana 6. Vgnda 7. Swazi 8. Shangaan 9. Ndebele
10 English 11. Afrikaans 12. Other

22 January 2010

8.2 Appendix 2: Antiviral drug usage questionnaire

Study number: _____

Cancer Epidemiology Research Group **Case-Control Study**

Antiretroviral Drug Usage

*Please ask **ALL PATIENTS** the following questions AFTER you have completed the standard questionnaire. Make sure that you emphasize that the information is voluntary and confidential.*

Have you ever been tested for HIV (before current test)? **Yes** $_1$ **No**$_0$ (circle one)

If YES, proceed: Were you tested**: before this illness**$_1$ / **at time of current illness only**$_2$ (circle one)

Were you given the test results? **Yes**$_1$ **No**$_0$ (circle one)

If YES, proceed: Are you willing to disclose your status? **Yes**$_1$ **No**$_0$ (circle one)

If YES, proceed: Are you HIV: **positive**$_1$ **negative**$_0$ (circle one)

Date of last/current test (mm/yyyy): __________

If POSITIVE, proceed:
Have you ever taken anti-Retroviral medicines for your HIV infection? **Yes**$_1$ **No**$_0$ (circle one)

(Be careful to explain to the patient that we mean "Western" medicine/pills/capsules specifically for the HIV itself, NOT for opportunistic infections.)

If YES, Proceed:

From: _____ (start date) To: _____ (end date)

From: _____ (start date) To: _____ (end date)

From: _____ (start date) To: _____ (end date)

The ARV drugs were provided by (tick all that apply):

☐ Private practitioner$_1$ ☐ Public hospital/clinic$_2$

☐ NGO$_3$ ☐ Study/ clinical trial$_4$

8.3 Appendix 3: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Melitah Motlhale

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M191130

NAME: (Principal Investigator)	Miss Melitah Motlhale
DEPARTMENT:	School of Public Health South African National Cancer Registry (Housed at National Laboratory Service's National Institute for Communicable Diseases)
PROJECT TITLE:	The role of HIV-1 on herpesvirus-related cancers in the adult (18 - 74 years) Black South African population
DATE CONSIDERED:	29/11/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Elvira Singh and Prof Freddy Sitas
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	29/11/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **November** and will therefore be due in the month of **November** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 09.12.19

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

8.4 Appendix 4: Paper 1-Epidemiology of Kaposi's sarcoma in sub-Saharan Africa

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Epidemiology of Kaposi's sarcoma in sub-Saharan Africa

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ARTICLE INFO

Keywords:
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Kaposi's sarcoma-associated herpesvirus
Antiretroviral therapy
Human immunodeficiency virus/acquired immunodeficiency syndrome
Sub-Saharan Africa

ABSTRACT

Kaposi's sarcoma (KS) has become a common AIDS-defining cancer in sub-Saharan Africa. Kaposi's sarcoma-associated human herpesvirus strongly modulated by HIV-related immune suppression are the principal causes of this cancer. No other risk factors have been identified as playing a strong role. HIV prevention programs and good coverage of antiretroviral therapy (ART) in developed countries resulted in a remarkable decline in HIV-KS incidence and better KS prognosis. By contrast, in sub-Saharan Africa, population ART rollout has lagged, but clinical studies have shown positive results in reduction of KS incidence and better KS prognosis. However, the effect of ART rollout in relation to population KS incidence is unclear. We describe the incidence of KS in sub-Saharan Africa, in four time-periods, (1) before 1980 (before HIV/AIDS era); (2) 1981–2000 (early HIV/AIDS era, limited or no ART coverage); (3) 2001–2010 (early ART coverage period); and (4) 2011–2016 (fair to good ART coverage period). We used KS incidence data available from WHO-International Agency for Research on Cancer (IARC) publications and the Africa Cancer Registry Network. National HIV prevalence and ART coverage data were derived from UNAIDS/WHO. A rapid increase in KS incidence was observed throughout sub-Saharan Africa as the HIV epidemic progressed, reaching peak incidences in Period 2 (pre-ART rollout) of 50.8 in males and 20.3 per 100 000 in females (Zimbabwe, Harare). The overall unweighted average decline in KS incidence between 2000 and 2010 and 2011–2016 was 27%, but this decline was not statistically significant across the region. ART rollout coincides with a decline in KS incidence across several regions in sub-Saharan Africa. The importance of other risk factors such as reductions in HIV incidence could not be ascertained.

1. Background

Kaposi's sarcoma is a malignant neoplasm caused by infection with Kaposi's sarcoma associated herpes virus (KSHV) [1]. Prior to the advent of the HIV/AIDS epidemic, Kaposi's sarcoma (KS) was present in Sub Saharan Africa at a larger frequency than the rest of the world [2–4]. Until the 1980s, little focus had been accorded to KS, which was regarded as an indolent tumor with relatively slow clinical progression

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[5]. With the emergence of the HIV/AIDS epidemic, the clinical severity, incidence and mortality of KS increased significantly, having a profound impact in the region [5–7]. The impact included increases in incidence in younger age groups, a narrowing of the male-female ratio, and an increase in KS into regions where it was not endemic [8,9]. KS is now predominantly an AIDS defining malignancy and one of the commonest cancers in sub-Saharan Africa.

Epidemic KS has been described due to coinfection with HIV and KSHV. KSHV is a necessary cause of KS [5,10]. The seroprevalence of KSHV in Africa is relatively high (20–80%) [6,8,11]. Sub-Saharan Africa accounts for approximately 70% of the global burden of HIV infection [12,13]. Co-infection with HIV and KSHV greatly increases the likelihood of developing KS [14]. Being male and age are factors related with risk of KS where it affects older people in endemic and classical, younger people in epidemic KS. Studies have further hypothesized that (non-viral) demographic and lifestyle risk factors for all KS types include infection with helminths and alcohol consumption [14–26]. The role of ethnicity in the etiology of KS is geographically bound by latitude north and south of central-east Africa and seems to be even less relevant in the presence of HIV than it was for the development of the classical or endemic KS before the HIV pandemic [9]. In the absence of HIV infection, risk factors associated with endemic KS remain unclear, this includes the male to female difference [19].

In sub-Saharan Africa, antiretroviral therapy (ART) is used to manage HIV / and AIDS related conditions including epidemic KS [19]. Since the introduction of ART, KS clinical outcomes have improved significantly with a combination of ART and chemotherapy [27]. In sub-Saharan Africa, complete remission of KS has been reported in about 20–80% of patients who were ART compliant [28,29]. The median time for remission in patients with KS due to ART has been reported to be between three to nine months [30]. ART is a cost effective intervention in decreasing the burden of KS, and offers great potential for improvements on survival, and quality of life for those with HIV-associated KS [31]. ART has also been shown to be an important way to reduce the risk of developing KS among people living with HIV/AIDS (PLWHA) in high income countries both in clinical follow-up and population studies [32]. The HIV burden, late rollout of ART, socio-economic issues and intervention implementation challenges in sub Saharan Africa could possibly explain the differential intervention outcomes between high income countries [12,33]. Clinical studies from Kenya, Uganda and South Africa indicate that ART is effective in reducing the incidence of KS [15,28,32].

In most sub-Saharan African countries, public sector ART programs were rolled-out in the mid-2000s. By 2017 approximately 11.8 million (46%) people living with HIV or AIDS in sub-Saharan Africa were receiving treatment [27]. ART coverage estimates are regularly compiled by the most recent report received by World Health Organization (WHO) and / or the Joint United Nations Program on HIV/AIDS (UNAIDS). The ART coverage estimates are calculated using statistical modeling methods [34].

WHO updated the ART guidelines for adults and adolescents in 2009. All adults and adolescents, including pregnant women with HIV infection and a CD4 count at or below 350 cells/mm^3 were enrolled for treatment, regardless of whether or not they have clinical symptoms. Those with WHO clinical stage 3 or 4 were to start treatment, irrespective of CD4 cell count. Old guideline thresholds for treatment initiation was < 200 cells/mm^3 [35]. In 2016, WHO launched revised, more inclusive guidelines for ART eligibility [27]. Since 2017, all people living with HIV or AIDS are recommended to receive ART, irrespective of CD4 count [27,36]. Most sub-Saharan African countries are formally rolling out ART programs, and most programs are now widening eligibility criteria to cover a broader group of HIV infected individuals outside of hospital systems.

There have been several review papers on KS pre and post the advent of HIV/AIDS (circa 1980) covering incidence or relative frequency of KSHV, associated risk factors in sub-Saharan Africa countries, and descriptions of the epidemiological and clinical characteristics of different forms of KS [1,15,19,27,37]. One review in 2010 focused on the epidemiology and transmission of KSHV as well as the clinical characteristics and therapy of HIV-associated KS in sub-Saharan Africa, with concerns raised about ART population coverage [38]. However, the effect of ART rollout on KS in sub-Saharan Africa at a hospital or population level has not been systematically documented.

We hypothesized that ART rollout should cause a decline in population-based incidence of Kaposi sarcoma in sub-Saharan Africa by comparing KS incidence to time periods pre and post rollout of ART.

2. Methods

This is a synthesis of literature on KS incidence and trends in relation to ART rollout in sub-Saharan Africa. We used KS incidence data available from WHO-International Agency for Research on Cancer (IARC) publications and the Africa Cancer Registry Network (AFCRN). National HIV prevalence and ART coverage data were derived from UNAIDS/WHO. Data on ART were gathered per sub-Saharan Africa country together with available HIV prevalence and available KS incident data. We reviewed published English articles on KS and ART related studies to assess the incidence of KS and the effect of ART over time in sub-Saharan Africa. Countries from North Africa were excluded because their climate conditions and cultural backgrounds have been considered to be more Mediterranean than African [34]. Conference abstracts were excluded. This review reflects on KS trends pre- and post-ART in sub-Saharan Africa.

2.1. KS age standardised incidence rates

KS incidence and trend data were derived from sub-Saharan African cancer registry reports in IARC Cancer Incidence in Five Continents (CI5) Volumes I-XI, two IARC Monographs on Cancer in Africa [39–41] and cancer registry reports published by the AFCRN [42,43]. KS age standardized (world) incidence rates (ASIR) per 100 000 persons were summarized for four time-periods, (1) before 1980 (before HIV/AIDS era); (2) 1981–2000 (early HIV/AIDS era, limited or no ART coverage); (3) 2001–2010 (early ART coverage period) and; (4) 2011–2016 (fair to good ART coverage period). Cancer registry data published by IARC and AFCRN were classified as being population-based (i.e. representing reasonable quality cancer registries and a good indication of KS incidence thus appearing in CI5 series (CI5 Vol VIII (1993–1998) [39], CI5 Vol X (2003–2007) [40], and CI5 Vol XI (2008–2012) [41]) and those published by AFCRN (Cancer in Africa Epidemiology and Prevention (1960–2000) [42], Cancer in Africa (2001–2014)(45) and Cancer in sub-Saharan Africa AFCRN Vol III [43]). GLOBOCAN reports (GLOBOCAN 2008 and 2012 reports [34,44], which in sub-Saharan Africa interpolate several countries' data, were only used for regional summary statistics.

2.2. Adult (15–49 years) HIV/AIDS prevalence, ART rollout and ART coverage percentage

Adult HIV prevalence data were drawn from country specific UNAIDS/WHO epidemiological fact sheet 2004 updates [45]. ART rollout and coverage data were drawn from data provided by UNAIDS/WHO website [46,47].

2.3. Regions

Data are presented for each country and within five African regions as defined by the United Nations Population Division [34]. Northern (excluded here for reasons mentioned previously, and for not falling in sub-Saharan Africa), Eastern, Southern, Middle (Central) and West Africa.

2.4. KS trends

Trends of the ASIR of the incidence of KS over the four time periods were presented graphically for males and females (Figs. 1 and 2). Fig. 3 illustrates the relationship between ART coverage and decline in KS incidence between 2001 and 2010 (early ART coverage period) and 2011–2016 (fair to good ART coverage period).

3. Results

Registries from eight of the 46 sub-Saharan African countries appeared in CI5 volumes I-XI and AFCRN reports and they were classified as fairly good quality registry data. Cancer registration coverage per region was poor with one country reporting out of the eight countries in Southern Africa, four countries out of the 14 countries in Eastern Africa, no country reporting in Central Africa (eight countries) and three countries reporting out of 16 West African countries.

3.1. ART Coverage (2018)

ART coverage increased from late 1990s, early 2000s to 2018, 30 countries have high (above 50%) ART rollout rates by 2018. In 2018 the lowest ART coverage was reported in Madagascar (9% in 2018) and the highest in Namibia at 92% [46,47].

3.2. Adult HIV prevalence, and ART roll out

The adult prevalence of HIV varies across sub-Saharan Africa, with a prevalence of < 0.1% in Comoros and 27.3% in Eswatini in the year 2018. Southern Africa has the highest burden of HIV compared to other regions. The earliest national rollout of ART in sub-Saharan Africa was in 1998 (Senegal); most countries rolled out ART in early 2000s and later programs started in 2006 (Chad).

3.3. Reported KS ASIR incidence per 100 000 from before 1980–2016

Table 1 reports the countries that reported KS ASIR to CI5. Before 1980, two population-based registries; Zimbabwe (Bulawayo) and Uganda (Kampala) reported KS incidence data for this period. Between 1981 and 2000, six registries reported KS cases and were published in CI5: Malawi, France La Reunion, Uganda (Kyadondo), Zimbabwe (Harare), The Gambia, and Mali (Bamako). For the period between 2001 and 2010, seven registries reported KS cases and were published in CI5: South Africa (Eastern Cape), Malawi, Uganda (Kyadondo), Zimbabwe (Harare), The Gambia, Mali (Bamako) and Nigeria (Ibadan). Seven registries published KS cases in CI5 between 2011 and 2016: South Africa (Eastern Cape), France La Reunion, Uganda (Kyadondo), Zimbabwe (Bulawayo), Zimbabwe (Harare), The Gambia and Mali (Bamako).

Table 2 reports on KS age standardized incidence rates (ASIR) for sub-Saharan African countries for 4 time-periods (pre-1980, 1981–2000,

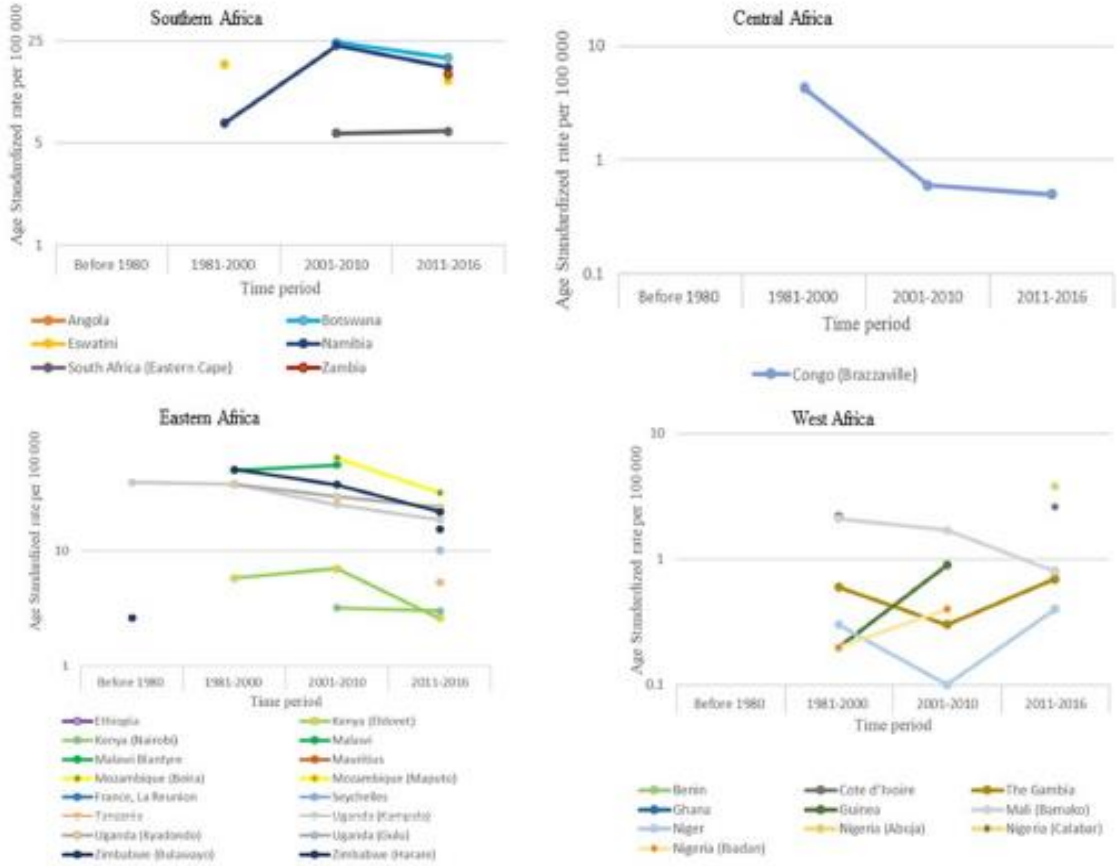

Fig. 1. KS ASIR per 100,000 trends in males (before 1980–2016) in sub-Saharan African countries. Cancer in Africa (2001–2014) [48]. Cancer in sub-Saharan Africa AFCRN Vol III [43]. Population based cancer registries. CI5 Vol VIII (1993–1998) [39]. CI5 Vol X (2003–2007) [40]. CI5 Vol XI (2008–2012) [41]. Data source: Cancer in Africa Epidemiology and Prevention (1960–2000) [42].

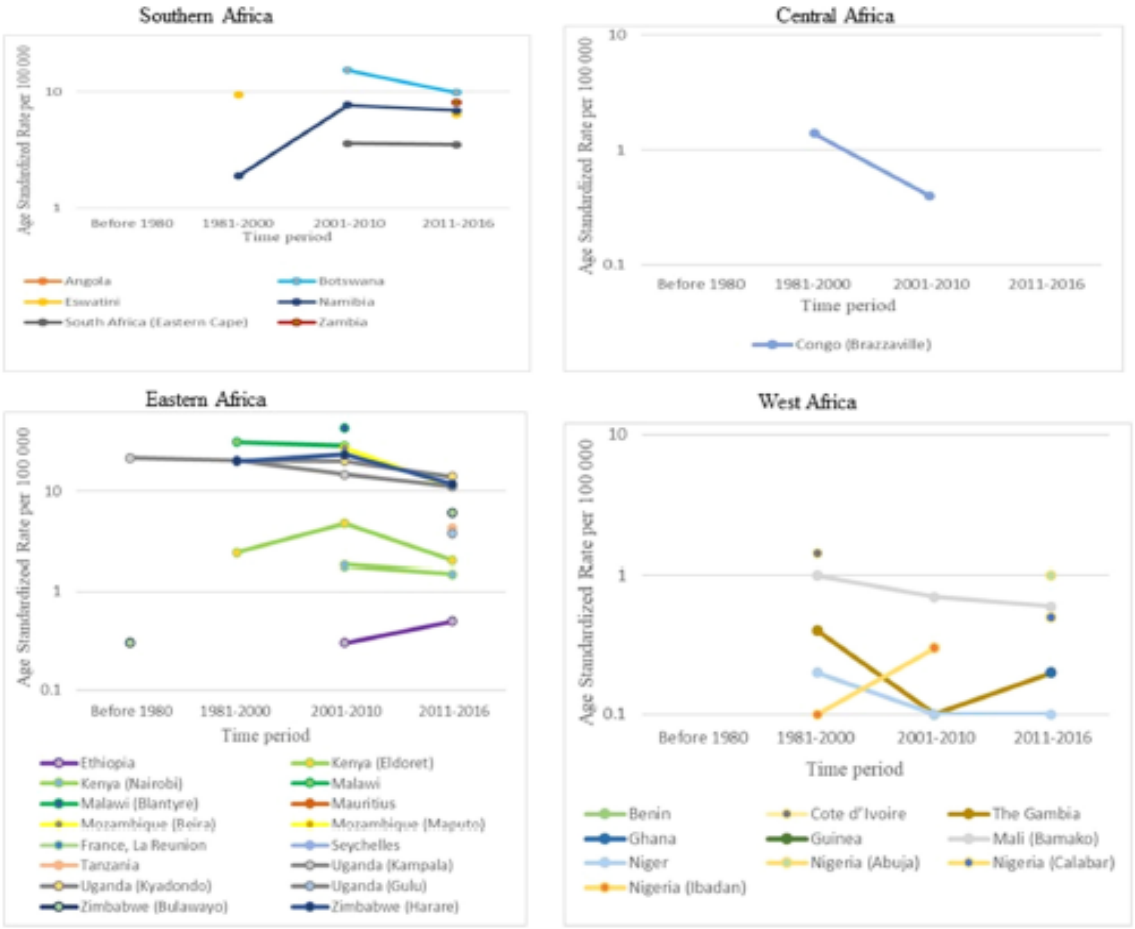

Fig. 2. KS ASIR per 100,000 trends in females (before 1980–2016) from sub-Saharan African countries. Cancer in Africa (2001–2014) [48]. Cancer in sub-Saharan Africa AFCRN Vol III [43]. Population based cancer registries. CI5 Vol VIII (1993–1998) [39]. CI5 Vol X (2003–2007) [40]. CI5 Vol XI (2008–2012) [41]. Data source Cancer in Africa Epidemiology and Prevention (1960–2000) [42].

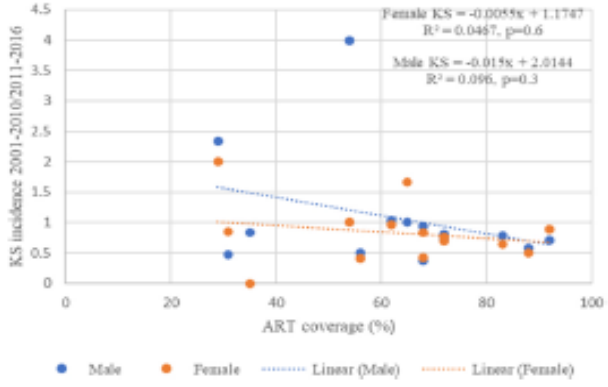

Fig. 3. Relationship between ART coverage and decline in KS incidence between 2001 and 2010 (early ART coverage period) and 2011–2016 (fair to good ART coverage period). Cancer in Africa (2001–2014) [48]. Cancer in sub-Saharan Africa AFCRN Vol III [43]. Population based cancer registries. CI5 Vol VIII (1993–1998) [39]. CI5 Vol X (2003–2007) [40]. CI5 Vol XI (2008–2012) [41].
Data source: Cancer in Africa Epidemiology and Prevention (1960–2000) [42].

Table 1
Countries that reported KS ASIR to CI5 pre- and post- HIV/AIDS epidemic.

Country ASIR published in CI5	Pre- and post- HIV/AIDS epidemic			
	Before 1980	1981–2000	2001–2010	2011–2016
Zimbabwe	✓	✓	✓	✓
Uganda	✓	✓	✓	✓
Malawi		✓	✓	
France La Reunion		✓		✓
The Gambia		✓	✓	✓
Mali		✓	✓	✓
South Africa			✓	✓
Nigeria			✓	

2001–2010 and 2011–2016). Figs. 1 and 2 shows KS ASIR trends over the four time periods. KS incidence rates varied over 10–20-fold amongst countries, from 2.6 to 39.3 in males and 0.3–21.8 in females; pre-1980, 0.0–32.0 in males and 0.0–11.8 per 100 000 in females for 2011–2016. In the pre-1980 period, KS was so rare that it was often combined with skin cancer or other miscellaneous categories or not classified in cancer registry reports [39].

Table 2
The burden of KS in sub-Saharan African countries in the regions over pre- and post- HIV/AIDS epidemic ART rollout periods (<1980, 1981–2000, 2001–2010 and 2011–2016).

Sub-Saharan Africa regions / countries	Latest adult (15–49) HIV prevalence% (2018)[b]	Introduction of ART (Year)[c]	Latest estimated coverage of ART % (2018)[d]	Before 1980 KS ASIR per 100 000[e]		1981–2000 KS ASIR per 100 000[f]		2001–2010 KS ASIR per 100 000[g]		2011–2016 KS ASIR per 100 000[h,i]	
				Male	Female	Male	Female	Male	Female	Male	Female
Southern Africa[a]								**11.5**	**5.1**	**7.6**	**4.7**
Angola	2	2003	27			0.0	0.0				
Botswana	20.3	2002	83					24.3	15.4	19.1	9.9
Eswatini	27.3	2003	86			17.2	9.5			13.5	6.4
Lesotho	23.6	2004	61								
Madagascar	0.3	2003	9								
Namibia	11.8	2003	92			6.8	1.9	23.2	7.7	16.4	6.9
South Africa (Eastern Cape)	20.4	2004	62					5.8	3.6	6.0	3.5
Zambia	11.8	2002	78							14.8	8.1
Eastern Africa[a]								**14.9**	**6.8**	**15.1**	**7.6**
Burundi	1	2003	80								
Comoros	< 0.1	2005	79								
Eritrea	0.7	2005	51								
Ethiopia	1	2005	65					0.6	0.3	0.6	0.5
Kenya (Eldoret)	4.7	2003	68			5.8	2.4	7.0	4.7	2.6	2.0
Kenya (Nairobi)								3.2	1.8	3.0	1.5
Malawi	9.2	2004	78	NC	NC	49.9	31.7	55.5	28.9		
Malawi (Blantyre)								91.8	43.6		
Mauritius	1.3	2001	22			0.0	0.0	0.0	0.0	0.0	0.0
Mozambique (Beira)	12.6	2004	56					63.6	27.5	32.0	11.2
Mozambique (Maputo)										22.7	11.6
France, La Reunion		2004				0.6	0.0			0.2	0.0
Rwanda	2.5	2003	87								
Seychelles		2001						0.0	0.0	0.0	0.0
Tanzania	4.6	2004	71							5.3	4.2
Uganda (Kampala)	N/A	2004	72	39.3	21.8	37.7	20.5	25.2	14.8	18.6	11.2
Uganda (Kyadondo)						37.9	20.4	29.5	20.2	24.0	14.1
Uganda (Gulu)										10.1	3.7
Zimbabwe (Bulawayo)	12.7	2004	88	2.6	0.3					15.5	6.0
Zimbabwe (Harare)						50.8	20.3	37.3	23.5	21.8	11.8
Central Africa[a]								**4.1**	**0.6**	**1.2**	**0.4**
Cameroon	3.6	2003	52								
Central African Republic	3.6	2003	36								
Chad	1.3	2006	51								
Congo (Democratic Republic)		2002	57								
Congo (Brazzaville)	2.6	2003	35			4.3	1.4	0.6	0.4	0.5	0.0
Equatorial Guinea	7.1	2005	34								
Gabon	3.8	2002	67								
Sao Tome & Principe	N/A	2005	N/A								
West Africa[a]								**1.9**	**1.2**	**0.9**	**0.6**
Benin	1	2002	61							0.4	0.0
Burkina Faso	0.7	2003	62								
Cape Verde	0.6	2004	89								
Cote d'Ivoire	2.6	2004	55			2.2	1.4			0.7	0.5
The Gambia	1.9	2004	29			0.6	0.4	0.3	0.1	0.7	0.2
Ghana	1.7	2004	34							0.8	0.2
Guinea	1.4	2003	40			0.2	0.0	0.9	0.3		
Guinea-Bissau	3.5	2005	33								
Liberia	1.3	2004	35								
Mali (Bamako)	1.4	2004	31	NC	NC	2.1	1.0	1.7	0.7	0.8	0.6
Mauritania	0.2	2003	54								
Niger	0.3	2005	54	NC	NC	0.3	0.2	0.1	0.1	0.4	0.1
Nigeria (Abuja)	1.5	2002	53							3.8	1.0
Nigeria (Calabar)										2.6	0.5
Nigeria (Ibadan)						0.2	0.1	0.4	0.3		
Senegal	0.4	1998	63	NC	NC						
Sierra Leone	1.5	2005	41								
Togo	2.3		60								

[a]GLOBOCAN 2008 and 2012 reports [34,44].
[b,c]UNAIDS/WHO epidemiological fact sheet 2004 update [45].
[d]UNAIDS website and WHO website [46,47].
[e,f,g,h]Population based cancer registries; AFCRN reports. Cancer in Africa Epidemiology and Prevention (1960–2000) [42]. Cancer in Africa (2001–2014) [48]. Cancer in sub-Saharan Africa AFCRN Vol III [43].
[i]Population based cancer registries; CI5 reports. CI5 Vol VIII (1993–1998) [39]. CI5 Vol X (2003–2007) [40]. CI5 Vol XI (2008–2012) [41].
N/A: Not applicable
NC: KS not classified.

3.3.1. Southern Africa

In the Southern African region, there was a lack of data on incidence rates of KS pre-1980. In 2008, GLOBOCAN reported that the KS incidence rates (per 100 000) for Southern Africa were 11.5 and 5.1 among males and females, respectively. GLOBOCAN 2012, reported a decrease on KS incidence rates (7.6 and 4.7 among males and females respectively). High incidence rates were reported in Swaziland/Eswatini [1981–2000]; males 17.2 and females 9.5. The ASIR decreased to 13.5 among males and 6.4 in females by 2011–2016. In Namibia, the incidence rates were lower for the 1981–2000 period [6.8 males, 1.9 females], there was a pronounced increase in the 2001–2010 period [23.2 males, 7.7 females].

3.3.2. Eastern Africa

Before 1980, only two registries (Uganda and Zimbabwe) out of the 14 countries in Eastern Africa reported KS age standardized incidence rates to IARC. There are still large gaps in data availability with no data reported for some countries. GLOBOCAN 2008 and 2012 reports indicate that this region shoulders most of the KS burden in sub-Saharan Africa. For 2001–2010, the ASIR for males was 14.9 and 6.8 for females and the rates increased to 15.1 for males and 7.6 for females [2011–2016]. Mozambique and Zimbabwe reported high incidence rates. In Mozambique (Beira), the ASIR for KS was 63.6 for males and 27.5 for females [2001–2010] and decreased to 32.0 for males and 11.2 females [2011–2016]. In Zimbabwe (Harare), the KS incidence rate was high 50.8 for males and 20.8 for females [1981–2000]. The reported rates decreased to 37.3 for males and 23.5 for females [2001–2010] and 18.4 for males and 10.3 for females [2011–2016].

3.3.3. Central Africa

GLOBOCAN reported that the KS ASIR for Central Africa were 4.1 and 0.6 among males and females respectively in 2008. GLOBOCAN 2012, reported a decrease on KS incidence rates (1.2 and 0.4 among males and females respectively). The only country that reported over all time periods was Congo (Brazzaville) and was characterized by a decrease in KS incidence rates over time. KS incidence varied from 4.3 for males and 1.4 for females [1981–2000] to 0.6 for males and 0.4 for females [2001–2010] and during 2011–2016 the ASIR for males was 0.5 and 0.0 for females.

3.3.4. West Africa

This region reported the lowest KS ASIR and the rates decreased over time. The ASIR was 1.9 for males and 1.2 for females [2001–2010] and the rates decreased to 0.9 for males and 0.6 for females [2011–2016] according to the GLOBOCAN reports in 2008 and 2012. Mali (Bamako) reported KS rates ranging from 2.1 for males and 1.0 for females [1981–2000] to 1.7 for males and 0.7 for females [2001–2010] and during 2011–2016 the ASIR declined for males to 0.8 and to 0.6 for females.

In summary, in the 16 registries from 14 countries that had sufficient serial data on KS, two showed no increase or decrease, 10 showed a decline and four showed an increase in KS incidence between 2000 and 2010 (early ART coverage period) and 2011–2016 (fair to good ART coverage period). GLOBOCAN reports on regional KS incidence estimates which vary from country to country within each region. With the understanding that these data are limited, incomplete and "ecological", Fig. 3 illustrates the geographical association between percentage ART coverage (latest data available in 2018) and KS incidence in the early vs. more recent ART periods. The overall unweighted average decline in KS incidence between the early ART and later period was 27%, but this decline was not statistically significant across the region. Fig. 4 illustrates the relationship between the latest adult (15–49) HIV prevalence (%) and KS incidence between 2011 and 2016. There was a moderate positive correlation between HIV prevalence and KS incidence for males and females.

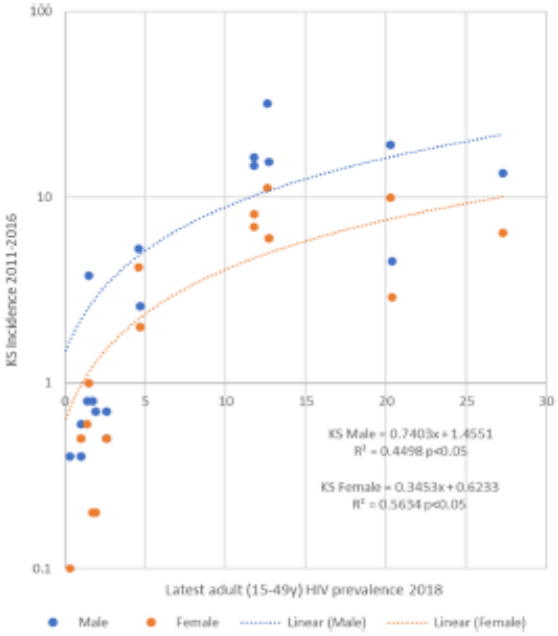

Fig. 4. Relationship between latest adult (15–49) HIV prevalence and KS incidence between 2011 and 2016. Cancer in Africa (2001–2014) [48]. Cancer in sub-Saharan Africa AFCRN Vol III [43]. Population based cancer registries. CI5 Vol VIII (1993–1998) [39]. CI5 Vol X (2003–2007) [40]. CI5 Vol XI (2008–2012) [41].
Data source: Cancer in Africa Epidemiology and Prevention (1960–2000) [42].

4. Discussion

4.1. Data quality and coverage

There is a significant under-reporting of cancer incidence in sub-Saharan Africa as there are few population-based cancer registries in the region. Cancer registration has been progressing slowly since the 1980 s and this can be attributed to several factors like insufficient and poor coordination of data sources, lack of adequately trained staff, poor infrastructure and low to no political will in other countries [42,49,50]. The KS incidence data used is from WHO-IARC and from AFCRN publications. These are considered to be reliable data source and cover about 24 countries – however not all of the data reported in the AFCRN are as strictly quality assured as in CI5. Prior to the onset of the HIV/AIDS epidemic in the 1980 s there were clear geographical variations in KS incidence within Africa. These are not obvious from sparse, high quality incidence data but previous work using relative frequencies does illustrate wide variations between central & East Africa compared to areas north and south [51].

4.2. The effect of ART on KS incidence in sub-Saharan Africa

During periods 2001–2010 and 2011–2016, 11 out of 16 registries with sufficient trend data showed a decline in KS incidence. Changes in KS incidence between these two periods vary from a 63% decline (Kenya, males) to a 400% increase (Nigeria, males), a crude average decline of 6%. KS trend data from a similar period (2000–2016) from the USA show a decrease in incidence of 35% [52].

The decrease in KS incidence has been small, this was not associated with ART coverage as measured [7,32] and possibly more related to broader changes in the HIV prevalence in many sub-Saharan African

countries over the years. One study reported that between 2000 and 2017, the estimated country level HIV prevalence increased in 15 of the 46 sub-Saharan African countries, unfortunately changes in HIV prevalence are not necessarily linear or consistently in the same direction in the region [53]. Another study reported a decline in HIV infections of more than 33% from an estimated 2.2 (2.1–2.3) million in 2005–1.5 (1.3–1.6) million in 2013, ART scale up and wide coverage were attributed to the substantial declines in new HIV infections [12].

Despite ART scale up in African countries HIV/AIDS-associated KS burden remains high in sub-Saharan Africa due to ART coverage gaps and policy variation [27,54]. Total coverage and implementation of 'test and treat all' is still a challenge in many sub-Saharan African countries because of limited resources and logistical issues [27]. In sub-Saharan Africa, the availability of ART has not completely resolved the challenges of epidemic KS [1]. The availability of oncologic care services and the existing underlying comorbidities may also be contributing to the variations [28].

Some of the decline in KS incidence can be attributed to the introduction of ART in sub-Saharan Africa, in the early 2000s and a drop in the HIV prevalence over the years. This is substantiated by epidemiological studies in South Africa and Uganda that reported an increase in KS cases, as the HIV/AIDS epidemic progressed, followed by a subsequent decrease once ART was introduced [19,55,56]. Epidemic KS is an important public health problem in sub-Saharan Africa because of the high KSHV and HIV prevalence [35–37]. KSHV is common in sub-Saharan Africa countries like Cameroon and Uganda where KS was relatively frequent pre-1980, but the virus is also common in countries like Botswana and Gambia where KS was rare before the spread of HIV [57]. KS is more common in males. It is not clear why KS is much more frequent in males. This suggests the need for more studies on the co-factors in the etiology of KS [9], and ideally development of an effective KSHV vaccine.

5. Limitations

Only 2% of the continent's population was covered by cancer registries that complied with the International Agency for Research on Cancer (IARC) quality standards in 2014. This indicates significant lack of population-based registries in Africa [27,31]. We used population-based registry data for cancer incidence, HIV prevalence and ART coverage data to reflect population trends. We acknowledge that the data are geographical, and the role of ART may not be ascertained using such correlation methods, differences in data sources used and large variations in ARV rollout policies.

6. Conclusion

In sub-Saharan Africa, there is limited evidence of a decline in KS incidence after the rollout of ART in some countries. However, epidemic KS continues to be a major public health problem in terms of morbidity and mortality. There is a paucity of literature on the burden of KS, risk factors associated with KS and the impact of ART on KS. There is a need for direct measures of the incidence and risk factors for KS in sub-Saharan Africa and effects of treatments. These could then be used to inform interventions, prevention, and management policies.

The continued recording of high numbers of KS in sub-Saharan Africa contrasts with its decline in developed countries and could be attributed to the ART treatment gap in the sub-Saharan Africa. The long-term effect of ART on KS in resource-limited regions like sub-Saharan Africa still needs to be evaluated to inform prevention and management interventions to improve the overall outcome for individuals living with HIV in this region. This requires continued investment in research of HIV associated malignancies in sub-Saharan Africa [27].

CRediT authorship contribution statement

Melitah Motlhale: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. Freddy Sitas: Conceptualization, Validation, Resources, Supervision, Writing – review & editing. Elvira Singh: Conceptualization, Validation, Resources, Supervision, Writing – review & editing. Debbie Bradshaw, Wenlong Carl Chen, Mwiza Gideon Singini, Chantal Babb de Villiers, Cathryn M. Lewis, Manvita Muchengeti, Tim Waterboer, Christopher G. Mathew, Freddy Sitas and Elvira Singh and Robert Newton are the members of ERICA_SA collaborative group. All authors read and provided feedback (writing: review and editing) to improve the final version of the manuscript.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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8.5 Appendix 5: Paper 2-Lifestyle factors associated with sex differences in Kaposi sarcoma incidence among adult black South Africans: A case-control study

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Lifestyle factors associated with sex differences in Kaposi sarcoma incidence among adult black South Africans: A case-control study

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ABSTRACT

Kaposi Sarcoma (KS) is endemic in several countries in Southern and Eastern Africa, relatively rare worldwide but a leading cancer among people living with HIV. KS has always been more common in adult males than females. We assessed the prevalence of known cancer modifying factors (parity, hormonal contraceptive use in females, sex-partners, smoking and alcohol consumption in both sexes), and their relationship to KS, and whether any of these could account for the unequal KS sex ratios. We calculated logistic regression case-control adjusted odds ratios (OR_{adj}), and 95% confidence intervals (95%CI), between KS and each of the modifying factors, using appropriate comparison controls. Controls were cancer types that had no known relationship to exposures of interest (infection or alcohol or smoking or contraceptive use). The majority of the 1275 KS cases were HIV positive (97%), vs. 15.7% in 10,309 controls. The risk of KS among those with HIV was high in males (OR_{adj}=116.70;95%CI=71.35–190.88) and females (OR_{adj}=93.91;95%CI=54.22–162.40). Among controls, the prevalence of smoking and alcohol consumption was five and three times higher in males vs. females. We found a positive association between KS and heavy vs. non-drinking (OR_{adj}=1.31;95%CI=1.03–1.67), and in current heavy vs. never smokers (OR_{adj}=1.82;95%CI=1.07–3.10). These associations remained positive for alcohol consumption (but with wider CIs) after stratification by sex, and restriction to HIV positive participants. We

Abbreviations: ARV, antiretroviral; CI, confidence intervals; ELISA, Enzyme-linked Immunoassay; hCG, human chorionic gonadotropin; HIV, human immunodeficiency virus; IARC, International Agency for Research on Cancer; ICD-O, International Classification of Diseases for Oncology; IQR, interquartile range; KS, Kaposi's sarcoma; KSHV, Kaposi's sarcoma-associated herpesvirus; JCS, Johannesburg Cancer Study; ORadj, Adjusted odds ratios; OR, odds ratios; SSA, sub-Saharan Africa; USA, United States of America.

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found no evidence of interactions of smoking and alcohol by sex. Smoking and alcohol consumption may provide a possible explanation for the KS sex differences, given both exposures are more common in men, but confounding and bias cannot be fully ruled out. The role smoking and alcohol play in relation to viral loads of HIV/KSHV, differences in immunological responses or other genetic differences between males and females warrant further studies.

1. Introduction

Kaposi Sarcoma (KS) is endemic in several countries in Southern and Eastern Africa and relatively rare cancer worldwide, with approximately 34,000 cases and 15,000 deaths reported by GLOBOCAN in 2020. [1] KS remains the most common cancer among people living with the human immunodeficiency virus (HIV). [2,3] KS is classified as an AIDS-defining cancer and the risk of developing KS is about 50–134 fold higher among those individuals who are HIV positive in South Africa.[4,5] Regardless of HIV positivity, throughout Africa, adult males have always been at higher risk of KS than females. [6,7] In South Africa, between 1988 and 2017 the pathology-based National Cancer Registry reported that KS incidence rates doubled in males and increased about seven-fold in females. This resulted in a decline of the sex ratio from 7:1 (males versus females) in 1988–2:1 in 2017. [8–10] Even though the ratio has declined, most likely as a result of the nature of the HIV epidemic in SA which predominantly affects women, the KS sex disparities are still evident.

Kaposi sarcoma-associated herpesvirus (KSHV) is considered a necessary cause of KS. [11] Co-infection with HIV and KSHV greatly increases the likelihood of developing KS, [12] which has resulted in a large increase in KS incidence during the HIV/AIDS pandemic in SA. [10]In Africa, the seroprevalence of KSHV varies from 20% to 80%. [4,8, 12–17] A recent meta-analysis of KSHV prevalence showed no difference in KSHV seroprevalence between boys and girls in SSA but found KSHV to be about 30% higher in adult males than females, which may explain some of the sex differences observed. [7] In other studies conducted in Uganda, males were twice as likely to have detectable KSHV in blood compared to females, [18–20] however in South Africa, we observed no Male: Female differences in adult KSHV seroprevalence. [12] The more than two-fold KS sex differences suggest the importance of further unknown cofactors other than KSHV in the development of KS. [9].

There is a dearth of information on KS lifestyle risk factors that could explain these sex differences. [21] The role of immunity and genomic make-up is currently uncharted. Grulich and Kaldor in 1996, at the early stages of the HIV epidemic, suggested sex hormones (e.g. during pregnancy) were not an important factor in KS pathogenesis. [22] Male hormones (androgen and testosterone) have been reported to aid KSHV infection and female pregnancy hormone human chorionic gonadotropin (hCG) inhibit the development of KS. [23–27] The role of smoking as a risk factor for KS was suggested by Goedert in 2009 to be protective in classical and epidemic KS in Sicily and the United States of America (USA). [28–30] The role of alcohol as a risk factor for KS is inconclusive. [21] These studies were, all however, small.

With some exceptions, smoking and drinking prevalences are higher in men than in women. Males are more likely to be in the heavy drinking and smoking categories as compared to females in South Africa. Peltzer and colleagues reported 41.5% men compared to 17.1% women as current alcohol users from the 2008 South African national population survey, whereas for all ethnicities, hazardous/ harmful drinking in black males and females were 15.5% and 10.0% respectively. [31] Results from the South African Smoking and Death Notification Survey (2007, aged 35–74) show black males had a smoking prevalence four times (46.3%) higher than black females (11.1%). [32].

We used data from the Johannesburg Cancer Study (JCS) to assess whether the lower incidence of KS in females (or higher incidence in males) could be attributed to any of known cancer-associated lifestyle factors such as parity, hormonal contraceptive use, lifetime sex partners, urban/rural residence, education, smoking and alcohol consumption in a black South African population.

2. Methods

2.1. Study setting and design

The JCS is an epidemiological study of over 25,000 cancer patients established by the National Cancer Registry of South Africa in 1995, at the early stages of the HIV epidemic. Data collection continued during the antiretroviral (ARV) rollout in 2004 and ended in 2016. The study aim of the JCS described in Chen et al., [33] was to investigate the relative importance of known cancer risk factors in an African population. This study utilizes an established case-control design for cancer epidemiological studies, whereby cases are individuals with the cancer of interest and controls are individuals with other cancers that are not associated with the exposures under investigation. [4,12,34].

2.2. Study population

The JCS recruited in a consecutive fashion, cancer patients of any type mainly from southern Gauteng province, attending the medical and radiation oncology departments of the largest tertiary referral public hospitals in Johannesburg (mainly Charlotte Maxeke Johannesburg Academic hospital and associated referral centres). [33] Participants were aged 18–74 years, black, resident of South Africa, with a new (incident) diagnosis of cancer, who had given written or witnessed oral informed consent. Trained nurse interviewers used a standard questionnaire to interview participants in their preferred language (usually Zulu or Sotho). Participants were interviewed within six months of diagnosis and before receiving radio- or chemotherapy.

The questionnaire covered questions on the leading, emerging and suspected risk factors for cancer such as place of birth and residence, education, ethnicity (home language of parents), method of cooking and heating, smoking by type of tobacco and amounts smoked, snuff (sniffed tobacco) use, alcohol consumption by type and amount consumed, parity, use of oral and injectable contraceptives, number of sexual partners, basic occupations, and self-reported use of ART (since 2005). Interviewers collected peripheral blood samples for HIV testing and other analyses. The serum was used for serological screening for HIV on all samples and KSHV and Human papilloma virus (HPV) in selected samples in previous studies. [4,12] HIV antibody testing was conducted using the Vironostika (HIV Uniform II plus O) micro enzyme-linked immunosorbent assay (ELISA). Cancer types were mostly (>90%) ascertained by cytology/histology[4] and coded to their topography and morphology using the International Classification of Diseases for Oncology Version 3 (ICD-O3).

2.3. Ethics

The University of Witwatersrand Human Research Ethics Committee (Medical) approved the primary study and the current study (Clearance certificate number: M191130).

2.4. Data management

Fig. 1 shows the participants used in case-control selection. Cases for

this study were participants with a histologically confirmed diagnosis of KS (N = 1275). Controls were women and men drawn from the rest (N = 19,161) of the participants diagnosed with cancer types that have no known relationship to exposures of interest. We used the IARC 100th Monograph series and Schottenfeld and Fraumeni's Textbook (3rd and 4th editions), to assess cancer/exposure relationships to identify appropriate control cancer types. [11,33,35–37] Control selection and therefore sample size varied when assessing the effect of HIV (infection-related cancers excluded, N = 10,309 remained), smoking (smoking-related cancers excluded, N = 2170 remained), alcohol consumption (alcohol-related cancers excluded, N = 3090 remained), parity and hormonal contraceptive use (reproductive-related cancers were excluded, N = 6870 remained) (Fig. 1). A detailed list of cancers included in each control group and the sex distribution among cases and controls (Supplementary Table S1 and Table S2). To test the robustness of the smoking and alcohol-KS-ORs we performed a sensitivity analysis recalculating these ORs by removing one cancer type at a time from each of the control comparison groups. (Supplementary Fig. S1 and S2).

The explanatory variables included sex, age group at enrolment, year of interview, place of birth (by province), ethnicity, place of residence (urban/rural), education (none, secondary or tertiary) and number of sexual partners (0–1, 2–5, 6 or more), weekly alcohol consumption (none, light (1–7 drinks for females and 1–14 drinks for males), and heavy (>8 drinks for females and >14 drinks for males). Cut-offs for heavy drinking were set at 8 or more drinks per week for women, 15 or more drinks per week for men according to the Centers for Disease Control and Prevention alcohol factsheet. [38] A current smoker was defined as someone who stopped smoking 5 years before interview to minimize reverse causation, and we conservatively assumed 1 g of tobacco per unit of tobacco products (mainly cigarettes). In females only, parity was classified as 0–2, 3 or more children born alive, and contraceptive use was measured in relation to combinations of ever/never use of injectable or oral contraceptives. Period of use of antiretroviral therapy period (ART-period) was classified as pre-ART (1995–2004), early ART (2005–2009) and late ART period (2010–2016).

2.5. Statistical analysis

The descriptive summary of the socio-demographic characteristics of cases and controls are shown in Table 1. To assess whether the high M:F sex ratio was associated with any of the leading modifying factors, unconditional unmatched logistic regression models were fitted to calculate their odds ratios (ORs) and 95% CI in relation to KS overall (Table 2) and stratified by sex (Table 3). ORs were adjusted for important socio-demographic variables (sex-where appropriate, age group, year of interview, HIV status, number of sexual partners, education, place of residence and ART period). For alcohol consumption and smoking we assessed the effect of an interaction term between alcohol, smoking and sex using unmatched logistic regression models (Supplementary Table S5 and S6).

We performed a test for heterogeneity on categorical variables and a score test for trend in the ORs on ordinal categorical variables (Tables 2 and 3). To adjust for confounding associated with HIV, smoking and alcohol consumption analyses were also restricted to HIV positive cases and controls. For each set of case-control comparisons, we used appropriate control comparison groups, conscious that the sample size in each set of comparisons varied. The statistical analysis was done using STATA software version 15.0 (Stata Corp, College Station, Texas).

3. Results

The majority of the 1275 individuals diagnosed with KS were male, 675 (53.0%) (Table 1). Most of the KS cases were HIV positive (95.6% males and 96.7% females), with very little difference in HIV prevalence across all the demographic and lifestyle groups studied. The highest proportion of KS cases was between the ages of 25 and 34 years in females and 35–44 among males. The majority of the cases (95%) were from urban places of residence and had secondary/tertiary education level (72.4% males and 80% females). Males reported a median of six sexual partners (IQR=4–10) and females a median of four partners (IQR=3–5). Most males, (N = 430, 63.7%) vs females (N = 172, 28.7%) were in the heavy alcohol consumption category.

Of the 675 male KS cases, 13.6% (N = 92) males vs 2.5% (N = 15) of

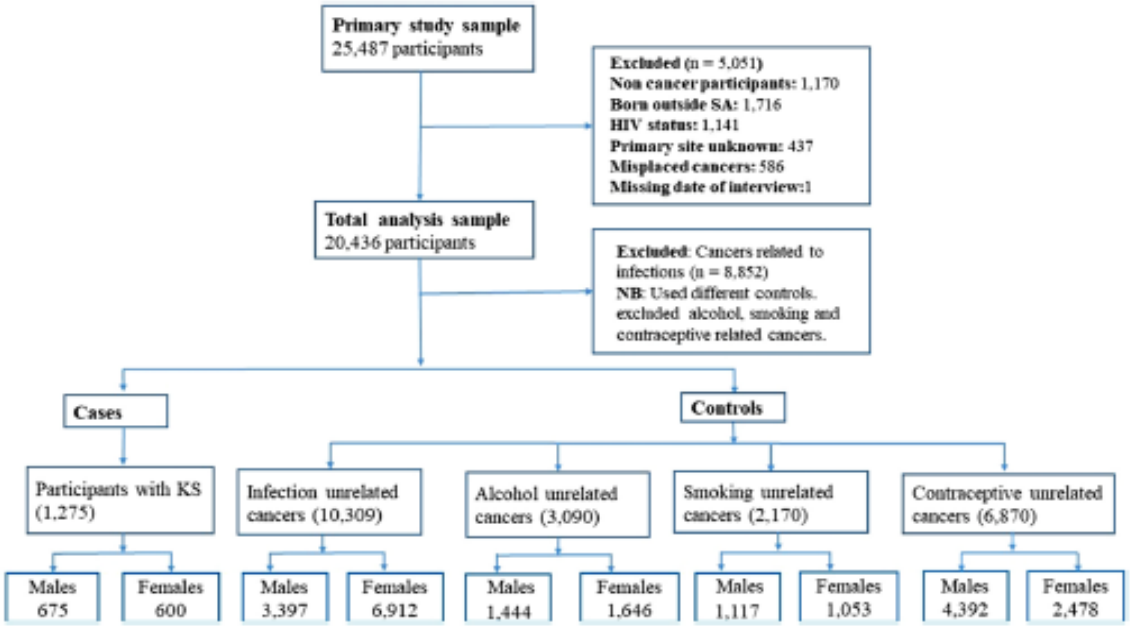

Fig. 1. Selection of study participants (cases and controls).

Table 1
Socio-demographic characteristics of study participants (cases and controls).

Characteristics	Cases (1275)		Controls (10,309[a])	
	Males	Females	Males	Females
	Total 675 (52.94) N (Col %)	Total 600 (47.06) N (Col %)	Total 3397 (32.95) N (Col %)	Total 6912 (67.05) N (Col %)
HIV status				
Negative	30 (4.44)	20(3.33)	3018 (88.84)	5674 (82.09)
Positive	645 (95.56)	580 (96.67)	379 (11.16)	1238 (17.91)
Age				
Median [IQR]	38 [33–44]	33 [29–40]	57 [49–64]	52 [43–61]
Age group (years)				
18–24	11 (1.63)	54 (9.00)	79 (2.33)	84 (1.22)
25–34	214 (31.70)	282 (47.00)	122 (3.59)	503 (7.28)
35–44	292 (43.26)	169 (28.17)	324 (9.54)	1395 (20.18)
45–54	120 (17.78)	69 (11.50)	887 (26.11)	1921 (27.79)
55–64	30 (4.44)	24 (4.00)	1197 (35.24)	1864 (26.97)
65+	8 (1.19)	2 (0.33)	788 (23.20)	1145 (16.57)
Period of interview				
1995–1999	41 (6.07)	33 (5.50)	827 (24.35)	1057 (15.29)
2000–2004	121 (17.93)	113 (18.83)	673 (19.81)	1217 (17.61)
2005–2009	229 (33.93)	229 (38.17)	895 (26.35)	1848 (26.74)
2010–2016	284 (42.07)	225 (37.50)	1002 (29.50)	2790 (40.36)
Place of residence				
Urban	647 (95.85)	564 (94.00)	3020 (88.90)	6187 (89.51)
Rural	26 (3.85)	34 (5.67)	362 (10.66)	697 (10.08)
Missing	2 (0.30)	2 (0.33)	15 (0.44)	28 (0.41)
Education				
None	31 (4.59)	24 (4.00)	462 (13.60)	740 (10.71)
Primary	155 (22.96)	96 (16.00)	1330 (39.15)	2023 (29.27)
Secondary & Tertiary	489 (72.44)	480 (80.00)	1596 (46.98)	4130 (59.75)
Missing	–	–	9 (0.26)	19 (0.27)
Number of sexual partners				
Median [IQR]	6 [4–10]	4 [3–5]	5 [3–9]	3 [2–5]
Number of sexual partners (cat)				
0–1	16 (2.37)	24 (4.00)	153 (4.50)	655 (9.48)
2–5	207 (30.67)	333 (55.50)	1319 (38.83)	3860 (55.84)
6 or more	265 (39.26)	115 (19.17)	1176 (34.62)	674 (9.75)
Unknown	187 (27.70)	128 (21.33)	749 (22.05)	1723 (24.93)
Alcohol consumption[b]				
Never	203 (30.07)	382 (63.67)	845 (58.55)	1368 (83.11)
Light > 0–7 or > 14 drinks/week	42 (6.22)	46 (7.67)	85 (5.88)	87 (5.29)
Heavy ≥ 8 or ≥ 15 drinks/week	430 (63.70)	172 (28.67)	514 (35.57)	191 (11.60)
Smoking[c]				
Never	206 (30.52)	483 (80.50)	393 (35.18)	891 (84.62)
Ex-smoker	69 (10.22)	20 (3.33)	273 (24.44)	71 (6.74)
Current-light 1–14 g/day	308 (45.63)	82 (13.67)	348 (31.15)	83 (7.88)
Current-heavy ≥ 15 g/day	92 (13.63)	15 (2.50)	103 (9.22)	6 (0.57)
Missing	–	–	–	2 (0.19)
Parity[d]				
0–2		372 (62.00)		866 (34.94)
3 or more		153 (25.50)		1436 (57.95)
Unknown		75 (12.50)		176 (7.10)
Contraceptive use[d]				
Never oral (oc) or injectable (ic)		152 (25.33)		1318 (53.19)
Ever oc never ic		49 (8.17)		271 (10.94)
Ever ic never oc		309 (51.50)		543 (21.91)
Ever ic and ever oc		88 (14.67)		332 (13.40)
#Ever ic and/ or ever oc		446 (74.33)		1146 (46.25)
Missing		2 (0.33)		14 (0.56)
ART-period				
Pre-ART 1995–2004	162 (24.00)	146 (24.33)	1500 (44.16)	2274 (32.90)
Early ART 2005–2009	229 (33.93)	229 (38.17)	895 (26.35)	1848 (26.74)
Late ART 2010–2016	284 (42.07)	225 (37.50)	1002 (29.50)	2790 (40.36)

Abbreviations: IQR, Interquartile range; oc, oral contraceptive; ic, injectable contraceptive.
#Ever ic and /or ever oc: a sum of category (2–4), ever oc never ic, ever ic never oc and ever ic and ever oc.
Total number of controls for the lifestyle exposures are different: for the demographic variables, infection unrelated cancer controls were used. For alcohol consumption, the controls comprised participants with cancers that were unrelated to infectious agents and alcohol. For smoking, the controls were not related to infectious agents and smoking. Contraceptive use controls were cancers unrelated to infectious agents and hormonal contraceptives.

[a] Infection unrelated controls– 10,309
[b] Alcohol unrelated controls– 3090
[c] Smoking unrelated controls– 2170
[d] Contraceptive unrelated controls– 6870

the 600 females smoked 15 g or more of tobacco per day (current heavy smokers). Most females, 372 (62.0%) reported giving birth to 0–2 children. More than half of the females, 309 (51.5%) reported only using injectable contraceptives and 49 (8.17%) reported only using oral contraceptives. Among the controls, HIV prevalence was 11.2% in males and 17.9% in females. About three times as many males (35.6%) reported heavy alcohol consumption (8–15 or more drinks a week) vs. females (11.6%). About five times as many males (40%) reported current smoking, vs. females (8%) (Table 1). Smoking and alcohol consumption prevalences are higher in males than females in both cases and controls. Socio-demographic characteristics of study participants by HIV status were also tabulated (Supplementary Table S3).

KS risk was highest among persons who tested HIV positive, OR_{adj} of 115.42 (95% CI=79.76–167.01). We found no association between KS and number of sexual partners (P_{trend}=0.40). Persons in the heavy drinking category were more likely to develop KS with an OR_{adj} of 1.31 (95% CI=1.03–1.67) compared to never drinking category (*P*

Table 2
Unadjusted and adjusted ORs (95% CI) of developing KS.

Variables	Unadjusted OR (95% CI) using appropriate controls	Adjusted OR (95% CI) using appropriate controls
HIV status		
Negative	1.00	1.00
Positive	131.70 (98.77 – 175.60)	**115.42 (79.76 – 167.01)**
P-value (heterogeneity)	< 0.001	< 0.001
Place of residence		
Urban	1.00	1.00
Rural	0.43 (0.33 – 0.56)	0.83 (0.54 – 1.28)
P-value (heterogeneity)	< 0.001	0.181
Education		
None	1.00	1.00
Primary	1.64 (1.21 – 2.21)	0.86 (0.56 – 1.32)
Secondary & Tertiary	3.70 (2.80 – 4.89)	0.86 (0.57 – 1.31)
P-value (heterogeneity)	< 0.001	0.876
Number of sexual partners		
0–1	1.00	1.00
2–5	2.11 (1.51 – 2.93)	1.26 (0.77 – 2.08)
6 or more	4.15 (2.96 – 5.81)	1.32 (0.79 – 2.21)
Unknown	2.57 (1.83 – 3.61)	1.24 (0.73 – 2.11)
P-value (trend)	< 0.001	0.402
Alcohol consumption[b]		
Never	1.00	1.00
Light	1.94 (1.47 – 2.54)	1.33 (0.87 – 2.04)
Heavy	3.23 (2.80 – 3.72)	**1.31 (1.03 – 1.67)**
P-value (trend)	< 0.001	< 0.001
P-value (sex contribution)		< 0.001
Alcohol consumption[b] (in HIV positives only)		
Never	1.00	1.00
Light	1.38 (0.91 – 2.11)	1.32 (0.83 – 2.09)
Heavy	1.86 (1.50 – 2.32)	**1.35 (1.04 – 1.76)**
P-value (trend)	< 0.001	< 0.001
Smoking[c]		
Never	1.00	1.00
Ex-smoker	0.48 (0.38 – 0.62)	1.00 (0.65 – 1.55)
Current-light (1–14 g)	1.69 (1.43 – 1.99)	**1.41 (1.03 – 1.92)**
Current-heavy (>= 15 g)	1.83 (1.38 – 2.43)	**1.82 (1.07 – 3.10)**
P-value (trend)	< 0.001	< 0.001
P-value (sex contribution)		0.013
Smoking[c] (in HIV positives only)		
Never	1.00	1.00
Ex-smoker	0.79 (0.52 – 1.18)	1.11 (0.68 – 1.81)
Current-light (1–14 g)	1.70 (1.28 – 2.26)	1.26 (0.90 – 1.77)
Current-heavy (>= 15 g)	2.47 (1.41 – 4.34)	1.84 (0.99 – 3.40)
P-value (trend)	< 0.001	0.087
Parity[d] (females only)		
0–2	1.00	1.00
3 or more	0.25 (0.20 – 0.30)	0.80 (0.59 – 1.09)
Unknown	1.00 (0.74 – 1.33)	0.83 (0.41 – 1.30)
P-value (trend)	0.9578	0.4590
Contraceptive use[d] (females only)		
Never oral or injectable	1.00	1.00
Ever oc never ic	1.57 (1.11 – 2.22)	1.48 (0.89 – 2.47)
Ever ic never oc	4.93 (3.97 – 6.14)	1.19 (0.86 – 1.64)
Ever ic and ever oc	2.30 (1.72 – 3.07)	1.25 (0.81 – 1.94)
Ever ic and/ or ever oc	3.37 (2.76 – 4.12)	1.24 (0.91 – 1.68)
P-value (trend)	< 0.001	0.3575
ART-period		
Pre-ART 1995–2004	1.00	1.00
Early ART 2005–2009	2.05 (1.76 – 2.38)	1.15 (0.89 – 1.48)
Late ART 2010–2016	1.64 (1.42 – 1.91)	0.83 (0.64 – 1.09)
P-value (trend)	< 0.001	0.175

[a] Demographic characteristics were compared to infection unrelated controls.
[b] Alcohol comparisons used alcohol unrelated controls
[c] Smoking comparisons used smoking unrelated controls
[d] Contraceptive use and parity analysis for females only using reproductive factor unrelated controls.
All ORs were adjusted for sex, HIV status, age group, year of interview, place of birth, ethnicity, place of residence, education, number of sexual partners and ART period.

$_{trend}$< 0.001). We found a strong statistical effect of sex in the overall model (Table 2, P_{value}< 0.001).

3.1. Additional analyses

After restricting the analysis to persons who were HIV positive (remaining N = 1225 KS, 1617 controls- i.e. losing approx. 80% of the controls) the odds of developing KS remained unchanged (OR_{adj}=1.35 (95%CI=1.04–1.76, P_{trend} <0.001)) in the heavy drinking category (Table 2). Those in the current heavy smoking category were more likely to develop KS (OR_{adj} 1.82 (95% CI=1.07–3.10, P_{trend}< 0.001) compared to never smoking (Table 2). We found a strong statistical effect of sex in the overall model (Table 2, P_{value}<0.013). Stratification of the data by sex is shown in Table 3. We found no evidence of sex*smoking (P-value 0.7) or sex*alcohol interactions (P-value=0.8) (Supplementary Table S5 and S6). We also tested the robustness of the smoking and alcohol KS associations by removing sex specific cancer controls from these analyses. We found no material differences in the ORs before and after removal (results not shown).

HIV positive males were similarly likely to develop KS as compared to HIV infected females (OR_{adj}=116.70; 95%CI=71.35–190.88 versus OR_{adj}=93.91; 95%CI=54.22–162.40) (Table 3). The association between smoking and KS remained positive in males for both smoking (P_{trend}=0.034) and alcohol consumption (p-trend=0.01). In females, elevated ORs were observed in the heavy drinking and smoking categories (OR=1.21 and 2.03) but the p-trend is no longer significant (P_{trend}=0.1, 0.6 respectively) perhaps because of reduced sample sizes.

4. Discussion

We attempted to identify potential lifestyle explanations for the well described excess male to female sex ratio in KS. We found that as in previous JCS study[4] being HIV positive increased significantly the risk of KS development. In contrast to some previous studies (see below) we found an association between increased smoking intensity and KS and heavy alcohol consumption and KS particularly in males. Smoking and alcohol consumption may provide a possible explanation for the KS sex differences. We found no association between KS and parity or hormonal contraceptive use, reconfirming the hypothesis stated by Grulich and Kaldor. [22] In a study estimating the prevalence of KSHV in females, de Sanjose and colleagues also reported that KSHV was not associated with the number of children, or patterns of oral contraceptive use. [39] Additional studies of the potential mechanisms for sex-related differences in KS are necessary to evaluate further the roles of sex-specific factors such as parity and female sex hormones in KS pathogenesis. [40].

As regards smoking, we found an association between increasing smoking intensity and KS, (Table 3). The association was evident in both sexes and in males alone, with sample sizes in females becoming being too low to draw clear inferences. The literature regarding the role of smoking is mixed. An inverse association between cigarette smoking and infection with KSHV or KS development has been reported in some studies, but these results were not adjusted for education and other confounding factors. [29,30,41] In one study in Uganda (N = 458 KS cases) and another in Cameroon (N = 266 cases), smoking was positively associated with HIV-related-KS; both studies adjusted for education and occupation associated with affluence. [42,43] Our study based on over 1000 KS cases and after adjustment for a range of confounders

Table 3
Unadjusted and adjusted OR (95%CI) of developing KS stratified by sex.

Variables	Unadjusted OR (95% CI)		Adjusted OR (95%CI)	
	Males	Females	Males	Females
HIV status[a]				
Negative	1.00	1.00	1.00	1.00
Positive	**171.21 (116.93 – 250.68)**	**132.91 (84.75 – 208.44)**	**116.70 (71.35 – 190.88)**	**93.91 (54.22 – 162.64)**
P-value (heterogeneity)	< 0.001	< 0.001	< 0.001	< 0.001
Alcohol consumption[b]				
Never	1.00	1.00	1.00	1.00
Light drinking	**2.06 (1.38 – 3.07)**	**1.89 (1.30 – 2.75)**	1.29 (0.68 – 2.46)	1.41 (0.79 – 2.50)
Heavy drinking	**3.48 (2.85 – 4.25)**	**3.22 (2.55 – 4.08)**	**1.46 (1.03 – 2.06)**	1.21 (0.85 – 1.72)
P-value (trend)	< 0.001	< 0.001	0.070	0.148
Alcohol consumption[b] **(HIV positives)**				
Never	1.00	1.00	1.00	1.00
Light drinking	1.00 (0.54 – 1.85)	1.73 (0.96 – 3.14)	1.08 (0.54 – 2.16)	1.63 (0.86 – 3.07)
Heavy drinking	**1.84 (1.33 – 2.57)**	**1.48 (1.07 – 2.05)**	**1.52 (1.04 – 2.23)**	1.21 (0.84 – 1.76)
P-value (trend)	< 0.001	0.012	0.034	0.100
Smoking[c]				
Never	1.00	1.00	1.00	1.00
Ex-smoker	**0.48 (0.35 – 0.66)**	**0.52 (0.31 – 0.86)**	0.87 (0.52 – 1.47)	1.49 (0.64 – 3.45)
Current-light	**1.69 (1.34 – 2.12)**	**1.82 (1.32 – 2.52)**	**1.51 (1.01 – 2.26)**	1.21 (0.72 – 2.04)
Current-heavy	**1.70 (1.23 – 2.37)**	**4.61 (1.78 – 11.96)**	**1.83 (1.00 – 3.35)**	2.03 (0.51 – 8.12)
P-value (trend)	< 0.001	< 0.001	0.010	0.621
Smoking[c] **(HIV positives)**				
Never	1.00	1.00	1.00	1.00
Ex-smoker	0.73 (0.44 – 1.24)	0.68 (0.31 – 1.49)	1.19 (0.65 – 2.17)	1.13 (0.46 – 2.76)
Current-light	**1.73 (1.15 – 2.61)**	1.17 (0.72 – 1.90)	1.48 (0.94 – 2.31)	0.97 (0.57 – 1.65)
Current-heavy	**2.14 (1.12 – 4.10)**	2.81 (0.64 – 12.43)	1.98 (0.98 – 3.99)	2.43 (0.52 – 11.26)
P-value (trend)	< 0.001	0.283	0.077	0.630

[a] For males and females, demographic characteristics used infection unrelated controls and adjusted for HIV status, age group, year of interview place of birth, ethnicity, place of residence, education, number of sexual partners and ART period.
[b] Alcohol using alcohol unrelated controls and adjusted for sex, age group, year of interview, education, HIV status, place of residence, number of sexual partners and ART period.
[c] Smoking using smoking unrelated controls and adjusted for sex, age group, year of interview, education, HIV status, place of residence, number of sexual partners and ART period.

including education, does support the hypothesis that smoking increases the risk of developing KS, and also given male smoker rates are about five times those of females, this could provide a possible explanation for the KS sex differences. Larger sample size studies, meta- analyses or cohort studies are needed to confirm this hypothesis as smoking makes a partial contribution to the KS sex difference.

While the initial analysis of the association between KS and alcohol is interesting, a crude 3-fold OR among those (in both sexes combined) in the heavy drinking category was reduced to 1.31 after adjustment, so significant confounding appears to be involved (Table 2). After stratifying by sex, those in the heavy drinking category (vs. non-drinking) were associated with increased KS risk in males but the association became weaker in females (Table 3). Restricting the analysis further to HIV positive participants indicated that heavy alcohol consumption was still associated with increased odds of KS in males and the effect being less important in females (although this could be due to reduced sample size). In this study, it seems plausible that alcohol consumption is another possible explanation for the KS sex ratios, especially given alcohol consumption is three times more prevalent in males. The relationship between KS and alcohol consumption is complex as the behavioral patterns associated with alcohol consumption may influence the probability of exposure to KSHV as stated by Mbulaiteye et al. [44] In a case-control study of KS in HIV positive Ugandan patients, Ziegler et al. and Nansseu et al. found no associated risk between alcohol consumption and KS. [42,43] KSHV seropositivity was also not associated with alcohol consumption in the study of Ugandan HIV-negative KS patients.[44,45].

Other explanations for the KS sex differences include genetic make-up along with unmeasured hormonal differences [46] and immune system functions.[47] Androgens aid KSHV infection and this may be why KS is more common in males than females. [27] Androgen receptor (found in both males and females) expression in males is high and has been identified as a KSHV entry factor and subsequently promotes KSHV infection in cells. [23,48] In females, the pregnancy hormone, human chorionic gonadotropin (hCG) has been reported to have shown anti-KS properties and this results in the reduction of KS tumor growth and inhibition of growth of other angiogenic tumors. [26] This hormone has also been shown to inhibit the growth of KS cell lines in vitro. [25] However, to the extent that we could measure (parity, hormonal contraception) we found no association between those factors in females and KS risk.

5. Limitations

Our study must be considered in light of possible limitations inherent in case-control studies and in this situation using people with a range of other cancers as controls. Cancer incidence in the South African black population is higher in females (National Cancer Registry data for 2017: 13,814 vs 13,116) and thus they are also more frequently represented in the JCS (7,512 vs 4,072). [10] This unusual situation, dominated by cancer of the cervix, distorts odds ratio calculations of M:F KS differences. In the analysis of risk factors, however, careful selection of controls unrelated to exposures of interest allows for valid comparisons. Population prevalence estimates for smoking and alcohol, or reproductive factors that are comparable with the 18–74-year-old black, urban population comprising the JCS study which collected data over a 20-year period, situated in southern Gauteng province are sparse. With that limitation in mind, national surveys[31] show prevalences of alcohol drinking of 35% in males and 10% in females with higher urban prevalences (in keeping with our observed prevalence of 41% and 17% in our mainly urban controls). Similarly, national current smoking prevalence in South African blacks is 50% in males and 12% in females, [32] in keeping with our observed 56% and 9% prevalence in our controls, HIV prevalence in Gauteng province is 14%, [39] again comparable to what

is observed in our controls (11% in males 18% in females).

Recall of past exposures such as number of sexual partners, alcohol consumption and smoking could be subject to recall or desirability bias. JCS collected data from radiotherapy and oncology departments and therefore, cases that were not referred to these departments (such as surgery only or palliative care) were missed. There may be possible residual confounding of lifestyle factors and HIV. Since 97% of all KS patients were HIV positive, there may be possible associations with high-risk behaviors such as number of sexual partners, smoking and alcohol drinking which are known to be associated with HIV, and the age pattern (reproductive age group). We accounted for HIV confounding by restricting the analysis for smoking and alcohol consumption to those who were HIV positive. Despite these limitations, these data remain an important source to understand sex disparities in KS among the adult black South African population.

6. Strengths

Both cases and controls are people with incident cancers, thus interviewer and recall bias is minimized (and is non-differential) by interviewing cases and controls with diseases of similar severity. Referral biases are also minimized because both cases and controls are likely to be come from the same catchment areas and referred through the same process and departments (as a precaution we adjust for Urban/Rural residence). Over 95% of the cancers were histologically verified. [4] Controls for this analysis were carefully selected, comprising cancer types not known to be associated with the exposures of interest (infectious agents, alcohol consumption smoking and hormonal contraceptive use). Sensitivity analysis of the controls used (Supplement Fig. S1 and S2) shows control selection was robust.

7. Conclusion

We have assessed, in a systematic fashion, whether selected lifestyles that are known to cause cancer are associated with KS development and whether these can explain the greater male to female KS ratio. In this study, being HIV positive increased the risk of KS in both sexes by 100-fold but the prevalence of HIV in controls was higher in females. compared to males (18 vs 11%). Parity and hormonal contraception use were not associated with KS among females. We found increased risks of KS (in both sexes combined) in relation to smoking and alcohol, and these risks remained positive after sex stratification and restriction to HIV positive participants. While confounding and biases cannot be fully ruled out, tobacco smoking and alcohol are therefore possibly responsible for the uneven KS sex ratios, given drinking and smoking was three to five times more common in males. While this is the largest case-control study to investigate these factors, reductions in sample size by restricting to HIV positives and by sex reduced our precision but the OR estimates remained about the same. Further comparative research, preferably from large cohort studies is needed to elucidate whether smoking and alcohol act independently or whether they modify the role of HIV and KSHV, the two viruses responsible for KS, especially their viral loads, immunological responses and in identifying other genetic differences in the way the two sexes respond to HIV/KSHV infection.

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Data sharing

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contribution

Melitah Motlhale, Freddy Sitas and Elvira Singh conceptualised the study. **Melitah Motlhale** performed data analysis, Writing – original draft; review and editing. **Freddy Sitas and Elvira Singh** validation, resources, supervision writing - review and editing. **Debbie Bradshaw, Wenlong Carl Chen, Mwiza Gideon Singini, Chantal Babb de Villiers, Cathryn M. Lewis, Mazvita Muchengeti, Tim Waterboer, Christopher G. Mathew, Freddy Sitas and Elvira Singh and Robert Newton** are the members of Evolving Risk Factors for Cancers in African Populations (ERICA-SA) collaborative group. All authors read and provided feedback (writing: review and editing) to improve the final version of the manuscript.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.canep.2022.102158.

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